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ORIGINAL ARTICLES

SOME OBSERVATIONS ON THE HAMMAN-RICH DISEASE*

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Twenty-five years ago, at the 52nd annual meeting of The American Clinical and Climatological Association, there was presented a paper by Louis Hamman and Arnold Rich in which they described 4 cases of diffuse interstitial fibrosis of the lungs which showed an extraordinary and distinctive pathological picture[6]. It is of particular interest that it was the similarity of the pathological lesions in these cases, more than of the clinical manifestations, which led the authors to group them together.

It was 9 years before Hamman and Rich found another case with similar characteristics, although their second publication in 1944 was again of 4 cases, one of the original 4 having been excluded as a questionable instance of this unusual type of pulmonary fibrosis[7].

By 1954, when Clough discussed this condition editorially in the Annals of Internal Medicine, he noted that a total of only 25 typical cases had been reported[2]. In the past 5 or 6 years many more cases, conforming in their pathological characteristics rather well with the original description, have been reported bringing the total to between 75 and 100 cases. Most of these differ, however, in some clinical respects from the originally described group, especially in regard to the duration of illness from the first symptomatic manifestations until death. The cases described by Hamman and Rich were all of relatively short duration, progressing rapidly to death within one to 6 months. The majority of subsequently reported cases have in contrast survived for an average of approximately 2 years, and one case, accepted by most students of the disease, survived without treatment for 9 years. Even the more slowly progressive cases, however, are frequently observed to pass from an indolent to a fulminating terminal phase, quite similar in symptomatology to the original cases. As is well known, the characteristic symptoms in this phase are of rapidly increasing dyspnea and cyanosis with death ensuing directly from pulmonary insufficiency, or from intractable cor pulmonale.

Most of the cases recorded in the literature were diagnosed only at necropsy, or the presumptive diagnosis was so confirmed. However, there is a grow-

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ing number of patients in whom this diagnosis has been made antemortem on the basis of lung biopsies.

Of the 3 cases to be described all were diagnosed antemortem, the diagnosis having been confirmed by postmortem examination in one and by lung biopsy in 2. The cases are presented not merely to add to the growing list but because they illustrate some of the problems which confront the clinician with respect to a disease which can no longer be regarded as extremely rare.

Case Reports. CASE 1. (Mr. H.B.): This patient, a man of 52, was admitted to the New York Hospital in October, 1954, for symptoms of cough, progressive dyspnea, weight loss and weakness for approximately a year, and of fever for 2 days. The symptoms initially had been mild, but the dyspnea and weakness became severe in the 2 weeks prior to admission. When fever was noted he was given penicillin for 2 days, without apparent benefit but this caused an urticarial skin eruption, which was the presenting complaint when the patient entered the hospital. He was acutely ill with temperatures ranging to 39° C. and respirations ranging from 30 to 40 per minute. Rales were widespread, but especially numerous at the lung bases. The chest roentgenogram showed a diffuse bilateral lower lung field infiltration, predominantly of soft confluent and patchy character but with an underlying reticular and nodular pattern. There was no response to antimicrobial therapy with tetracycline, or when this was changed to chloramphenicol and dihydrostreptomycin. Ten days after admission, cortisone therapy was begun, and this was continued in the dosage of 100 mg. daily, until exitus on the 31st hospital day. Chloramphenicol therapy was also maintained, but the dihydrostreptomycin was discontinued 10 days before death because of an elevation of the blood urea nitrogen, which nevertheless continued to rise terminally to 75 mg. per 100 ml. Despite extreme cyanosis, oxygen therapy was poorly tolerated, but there was no evidence of respiratory acidosis. There were no conspicuous manifestations of cor pulmonale or right heart failure. Death appeared to result primarily from respiratory failure.

The postmortem examination showed the lungs grossly to be firm and of rubbery consistency on cut section. In the basal segments of the lower lobes and in the right middle lobe particularly, the parenchyma was largely replaced by a matrix of fibrous tissue, in which were embedded numerous cystic spaces from 7 to 20 mm. in diameter, containing no fluid or other secretion and lined by a glistening surface. The microscopic findings are shown in Fig. 1. Here may be found most of the features described originally by Rich: a) the diffuse inflammation with edema, hemorrhage and fibrin deposition, but few leukocytes, b) the enlargement of the lining alveolar cells, c) the necrosis of alveolar and bronchiolar epithelium, d) the formation of a hyaline membrane, e) the extensive interstitial fibrosis (Masson's stain). The only important feature that is missing, and which was absent also in one of the 4 original cases, is the presence of eosinophils in the interstitial tissues.

The postmortem findings showed no associated diseases, except generalized atherosclerosis and a necrotizing glomerulitis and arteritis in the kidneys.

COMMENT. This patient's clinical history, with relatively mild symptomatic onset only one year before death, and a fulminating terminal phase lasting only a little over a month, resembles most nearly the course of the originally described cases. The failure of response either to antimicrobial or to steroidal hormone therapy reflects the common experience of those who have attempted to treat the disease in the acute terminal phase. Peabody, Buechner and Anderson first reported further that in several instances patients treated with corticotropin or with adrenocortical steroid hormones not only failed to benefit but died suddenly when these agents were discontinued or reduced[8]. In the present instance there is little to suggest that the cortisone either benefited the patient or precipitated death. The diagnosis of Hamman-Rich disease was made antemortem on clinical grounds alone. The patient was evidently in the terminal phase when seen and was too ill to permit a lung biopsy being performed. The pathological findings appeared

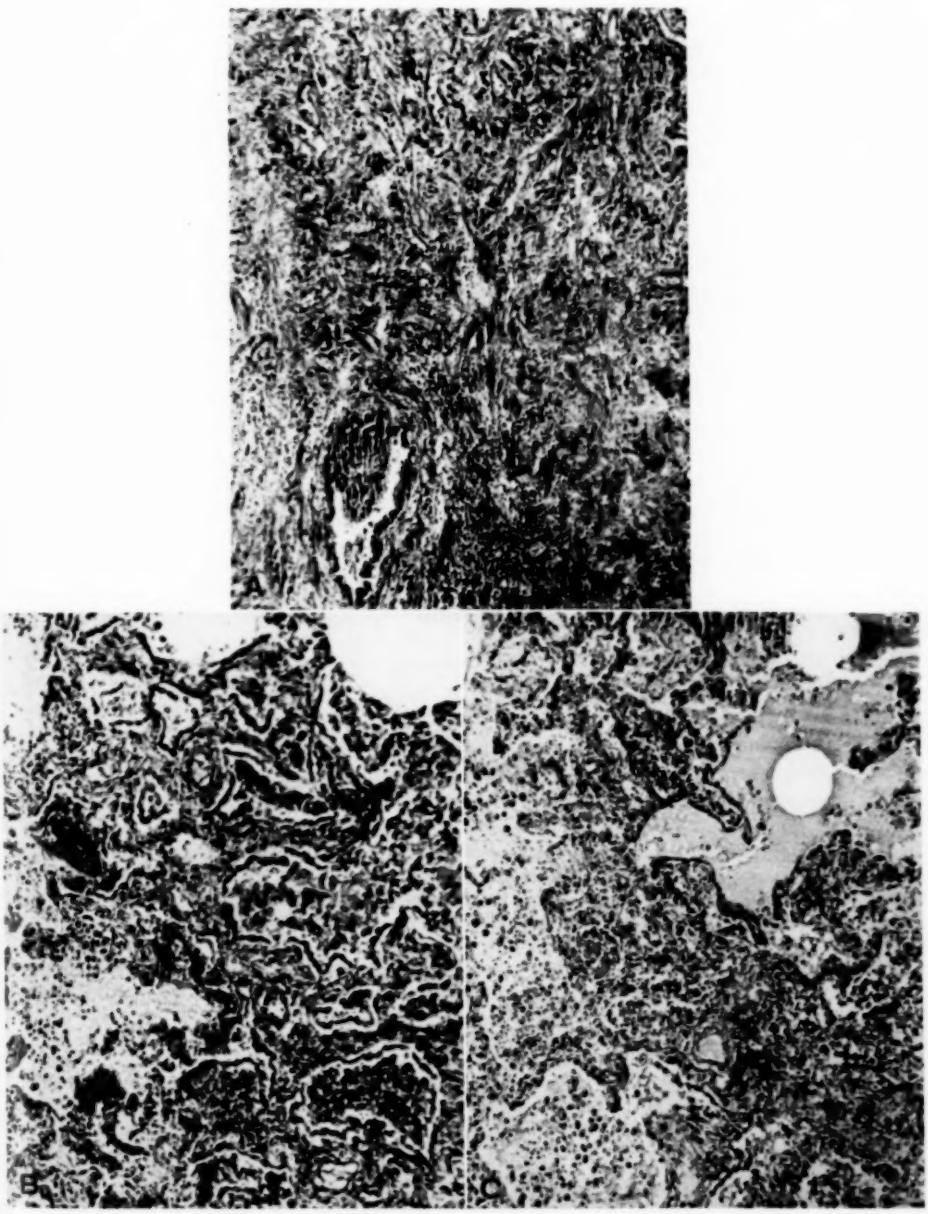

Fig. 1.—Case 1. Hematoxylin and eosin. Magnification 90×. *A*. Section from right lower lobe. *B* and *C*. Sections from right middle lobe. Note hyaline membrane in *C*.

to be confirmatory. The renal findings of glomerulitis and arteritis were thought by Dr. John Ellis, in his final review of the postmortem record, to be probably not related to the pulmonary disease but possibly to a drug hypersensitivity reaction to penicillin.

CASE 2. (Mrs. D.B.): This patient, a 39-year-old woman, was first seen in March, 1954, when she was admitted to the New York Hospital for the complaint of exertional dyspnea of approximately 5 years' duration. She had also a history of recurrent attacks of bronchitis since childhood and two episodes of possible pneumonia within the past 6 years. One year prior to admission a roentgenogram of the chest showed some light diffuse bilateral lung infiltrations. These had markedly increased at the time she entered the hospital, as had also the exertional dyspnea in the one year interval. The significant findings on physical examination were widespread crepitant rales, over the lower lung fields especially, and marked clubbing of the fingers. Cyanosis was not conspicuous. The vital capacity was moderately reduced, to 66% of normal, but the maximum breathing capacity was 120% of normal. Blood gas studies showed normal arterial oxygen saturation at rest but a marked unsaturation with exercise. There was normal carbon dioxide tension, both at rest and with exercise. The pulmonary functional disturbance was regarded by Dr. Daniel Lukas, who performed these studies, as characteristic of the alveolar-capillary block type of diffusion defect. A lung biopsy was performed by open thoracotomy. At operation, Dr. Cranston Holman observed the entire left lung to be considerably firmer than normal lung, with diffuse nodularity and many small emphysematous blebs. The biopsy was taken from one of the numerous, small, especially firm areas. This showed the parenchyma of the lung to be replaced by fibrous tissue with only a few remaining alveoli which appear as epithelial-lined spaces, giving the tissue an adenomatous appearance. The large basophilic cells which line the alveolar spaces vary considerably in size and shape, but were considered by the pathologist, the late Dr. John Pearce, to show no true anaplasia, such as might be seen in pulmonary adenomatosis. The intervening fibrous tissue is infiltrated with lymphocytes, plasma cells and eosinophils. Neutrophilic polymorphonuclear cells are scarce.

TABLE 1.—CASE 2. FUNCTIONAL IMPAIRMENT 2 YEARS AFTER CONCLUSION OF ONE YEAR COURSE OF CORTISONE THERAPY, AND 3 YEARS BEFORE DEATH

Mrs. D.B. *Pulmonary Function Studies (2-15-57)*

Vital cap. 1435 (47%)
after vaponefrin 46%
Max. bg. cap. 62 L./min. (72%)
after vaponefrin 53%
Arterial oxygen sat.
at rest—92.1%
exercise—79.8%
CO_2 tension
rest—39 mm. Hg
exercise—41 mm. Hg

Ventilation	*Rest*	*1st min. exercise*	*1st min. recovery*
	4.5 L./min.	12.5	18.2

Cortisone therapy was begun approximately one month postoperatively and maintained for approximately one year. The daily dosage was never above 75 mg. and for the greater portion of the course it was maintained at 50 mg. This dosage may have been inadequate, as there was no apparent improvement in either symptoms or signs. Neither was there any exacerbation when the cortisone was withdrawn. In the next 2 years there was little change in symptoms, but pulmonary function studies (Table 1) now showed a considerable further deterioration. Both the vital capacity and the maximum breathing capacity had declined (to 47% and 72% of normal respectively). It is interesting to note that after an epinephrine aerosol there was a decline rather than an increase in these

measurements, especially in the maximum breathing capacity. The arterial oxygen saturation was now slightly reduced at rest, and declined to below 80% with standard exercise. Carbon dioxide tensions remained normal. A marked increase in ventilation during and after exercise was noted, reaching a peak of four times the resting level by the first minute of the recovery period.

In early 1958 there was a noticeable increase of symptoms and a 3 months' course of prednisone (20 mg. per day) was given, again with no apparent benefit nor exacerbation upon its withdrawal.

In January, 1959, or nearly 5 years after the first hospital admission, there developed an episode of congestive failure for which the patient was admitted for the second time. The marked increase in pulmonary shadows, shown in a roentgenogram just prior to readmission, was apparently due to some degree of pulmonary vascular congestion, indicating an element of left ventricular failure accompanying the right ventricular failure of cor pulmonale. The heart was also markedly enlarged. These roentgenographic changes were largely reversed after treatment of the congestive failure which was accomplished without particular difficulty by digitalization, diuretics and repeated phlebotomies (the hematocrit having risen to 58%). After recovery from the bout of congestive failure, prednisone therapy was again instituted and maintained during the following year, beginning with 20 mg. per day for the first 6 months and then successively reduced to daily maintenance doses of 10 mg. and 5 mg. Cardiac compensation was maintained successfully for 8 or 9 months with maintenance doses of digitoxin, and was restored by vigorous diuretic therapy when a mild episode of failure was precipitated by an intercurrent upper respiratory infection. A third episode of congestive failure necessitated hospitalization again in January, 1960. The edema and ascites now proved to be refractory to the usual diuretic regimens, and also to the acidification regimen with lysine chloride and to a course of spironolactone. Repeated abdominal paracenteses were necessary to relieve the ascites. Finally an appreciable diuresis was induced by large doses of prednisone (80 mg. per day) and the patient improved sufficiently to be able to leave the hospital (March 2, 1960). Death occurred suddenly, however, a week afterward at home from what the family physician believed to be a coronary occlusion. A postmortem examination was not performed.

COMMENT. This patient's 6-year course, though by no means the longest on record, is illustrative of the more slowly progressive cases which, as already mentioned, comprise the majority of those more recently reported. The diagnosis in this instance was made early, by means of a lung biopsy. The patient finally died at home and no necropsy could be performed, but there is little reason to think that the biopsy material was not representative of the diffuse disease in the lungs. The microscopic findings reflect all, or nearly all, of the principal features described originally by Rich. The pulmonary function studies reflect the principal characteristics of the alveolar-capillary block syndrome, which is in essence what has been described in other patients with Hamman-Rich disease in whom the results of physiologic studies have been reported. The therapy with cortisone was undertaken with some hesitancy, because of the aforementioned observations of Peabody, Buechner and Anderson, and the few isolated reports of definite benefit from adreno-corticosteroid therapy had not yet then been published. It is possible that the dosage in the initial course was inadequate. The most that can be said of its efficacy is that it may have delayed somewhat the progression of the disease. It seems quite clear that the initial and subsequent course of adreno-corticosteroid therapy did no harm and that their termination produced no exacerbations. This is one of only a very few patients thus far described who survived for longer than a year following a first episode of severe congestive failure from cor pulmonale. The ultimate terminal phase of this patient's illness was characterized not by any features suggesting a fulminating progression of the underlying pulmonary disease but by the mani-

festations of intractable congestive heart failure.

CASE 3. (Mr. W.M.): This 56-year-old man was first seen in November, 1956, when he was admitted to the New York Hospital for a complaint of progressive exertional dyspnea of 10 years' duration. Physical examination was at this time unremarkable except for marked clubbing of the fingers with appreciable cyanosis of the nail beds, and for scattered rales over both lower lung fields. The roentgenogram of the chest showed interstitial fibrosis, principally in the lower lung fields, and prominence of the pulmonary artery shadows. The bilateral infiltration had been previously noted 1½ years earlier, but at this time it was less conspicuous and less extensive. There was no polycythemia and routine blood chemical and sputum studies were unrevealing. A scalene lymph node biopsy showed no pathology. A lung biopsy was performed, after preliminary studies of pulmonary function were completed. At operation the left lung was granular in appearance and to palpation, but no large nodules were seen. Microscopically the findings were similar to those of Case 2, showing the interstitial fibrosis, the cuboidal metaplasia of the cells lining the alveolar spaces, the scarcity of polymorphonuclear leukocytes and the presence of lymphocytes, plasma cells and eosinophils (Fig. 2). The extent of fibrosis is particularly well seen in sections stained by the Masson technique (not reproduced).

Postoperatively there was begun a course of prednisone therapy and this was continued for 5 months. As there was no apparent im-

Fig. 2.—Case 3. Hematoxylin and eosin. Magnification 90×. Biopsy section from left lower lobe.

provement symptomatically, roentgenographically or in the physiological tests (which were repeated 4 months postoperatively) the therapy was discontinued. Six months after the end of prednisone therapy the patient thought he was slightly less dyspneic and that the finger clubbing and nail-bed cyanosis had receded. This subjective improvement was not reflected, however, by objective function studies which were again repeated at this time.

TABLE 2.—CASE 3. PULMONARY FUNCTION STUDIES BEFORE LUNG BIOPSY, 4 MONTHS AFTER LUNG BIOPSY AND ONE YEAR AFTER LUNG BIOPSY. THE PATIENT RECEIVED PREDNISONE THERAPY FOR 5 MONTHS, COMMENCING ONE MONTH AFTER THE OPERATION. NORMAL VALUES FOR VENTILATION, ARTERIAL OXYGEN SATURATION AND CARBON DIOXIDE TENSION ARE SHOWN BETWEEN PARENTHESES

Mr. W.M.	*Pulmonary Function Studies*					
	Pre-op. (lung biopsy)		*4 mos. post-op. (during Prednis.)*		*1 yr. post-op. (6 mos. post-Rx)*	
Vital Cap.	normal		normal		normal	
M.B.C., % normal	85		98		107	
after vaponefrin	94		117		117	
		Predicted				
Ventilation,, L./min./M²	6.8	(3.9)	9.0		7.3	
during exercise	21.0	(11.2)	25.2		27.7	
1st min. recovery	24.7	(14.5)	35.4		37.3	
2nd min. "	17.2	(10.8)	31.6		—	
5th min. "	14.9	(6.3)	22.1		27.9	
Arterial Oxy. Sat., %	97.3	(97)	94.4		89.2	
after exercise	78.6	(97)	79.2		80.8	
CO_2 tension, mm.Hg	40	(39)	29	(39)	—	
after exercise	35	(38)	29	(40)	—	

It is apparent (Table 2) that the marked increase in ventilation and decrease in arterial oxygen saturation with exercise were unimproved, both during the prednisone therapy and 6 months after its conclusion. Neither was there at this time (1 year after the initial studies), nor a year later any evidence of roentgenologic improvement. The patient has been followed-up with periodic physical and roentgenographic examinations to the present with no further changes except that rales have been usually absent since the course of prednisone therapy and the finger-clubbing, as first noted by the patient himself, has seemed to be less conspicuous.

COMMENT. This patient's disease, which, when he was first seen, was manifestly progressive, both symptomatically and by physical and roentgenographic signs, has become apparently stabilized. Whether this stabilization can be attributed to the single course of steroid hormone therapy which he received is uncertain. It is of interest, nevertheless, that whether beneficial or not the therapy and its withdrawal were conducted without apparent harm and the apparently stabile clinical and roentgenologic status have persisted for more than 2 years without any further therapy.

Discussion. There is no need nor occasion to review here in detail the by now extensive literature on this subject nor attempt to analyze the published cases of diffuse interstitial pulmonary fibrosis considered to resemble the clinical and pathological entity described by Hamman and Rich. This was done in 1956 by Grant, Hillis and Davidson[4], in 1957 by Rubin and Lubliner[10] and in 1960 by Baglio, Michel and Hunter[1]. The fundamental question to which these authors have addressed themselves, as have others

who have published more than isolated case reports, is whether the apparent pathologic unity among cases of considerable clinical disparity actually reflects a disease entity with a common, though as yet undetermined etiology; or whether this represents rather a nonspecific type of reaction to a variety of disease processes. Resemblances have been pointed out between the pathological findings in cases conforming clinically to what is most usually termed the Hamman-Rich syndrome and in cases of interstitial fibrosis associated with manifestations of other more or less well-defined systemic diseases. Rubin and Lubliner[10] have emphasized particularly the resemblance to the pulmonary findings in some cases of rheumatoid disease. Similarities have been noted to pulmonary lesions found in other groups of collagen disease, such as dermatomyositis and scleroderma. Indeed, the possibility has been considered that Hamman-Rich disease is essentially a collagen disease, limited to the pulmonary organ system. Further similarities have been noted to certain phases of viral pneumonias, and to the interstitial pulmonary lesions found in patients treated for hypertension with such agents as hydralazine and hexamethonium. Similarities to the Hamman-Rich type of interstitial pulmonary disease have been remarked also in such conditions of known etiology as berylliosis and *Pneumocystis carinii* pneumonia. These several similarities have influenced the various theories that have been advanced relative to the etiology of the disorder. These have been grouped under three principal categories, namely 1) the infection theory, 2) the hypersensitivity theory, and 3) the collagen disease theory, including the rheumatoid theory. The possibility of a familial factor was first noted by Peabody, Peabody *et al.*[9], who observed the occurrence of the disease in identical twins. The occurrence of the disease in siblings has since then been reported as relatively frequent. Recently Donohue *et al.* have attempted to relate Hamman-Rich disease to a familial fibrocystic pulmonary dysplasia and claim some 25% of published cases of the Hamman-Rich syndrome to be of congenital origin[3]. In this, however, these authors have admittedly broadened the definition and included some cases which they found in the literature under other designations. A final comment on the recent literature concerns a claim by Grosse-Brockhoff[5] that a condition described by Rindfleisch in 1897 as cirrhosis of the lung, and later by other German pathologists, is essentially the same condition as that which was here described in 1935 by Hamman and Rich, though giving the latter credit for more fully characterizing the disease. I think that Rich is himself best qualified to determine whether these earlier cases were of a similar nature, and also whether the 3 cases which have been here presented properly fall into the category of a disease, or syndrome as it is more usually designated, which has come to bear his name. The 2 papers which he published with Hamman on this subject bear the titles respectively of "Fulminating Diffuse Interstitial Fibrosis of the Lungs"[6] and "Acute Diffuse Interstitial Fibrosis of the Lungs"[7]. I must admit, therefore, a certain presumption in presenting these 3 cases as instances of the same disease, since in none of the 3 was the condition quite as fulminating or acute. The alternative would be to diagnose these cases merely as nonspecific diffuse interstitial pulmonary fibrosis, pointing out parenthetically that the pathological and some of the clinical features bear some resemblance to the disease described originally. That there

is a group which can be clinically and pathologically differentiated from such other diffuse pulmonary conditions as sarcoidosis, berylliosis, the pneumoconioses, the systemic collagen diseases with pulmonary involvement, the pulmonary complications of hypotensive therapy and other fibroses of known etiology seems to be well established by the growing number of cases reported in which none of these factors are in evidence. The special problems of clinical management, especially in relation to therapy with the adrenocorticosteroid hormones, make the clinical diagnosis a matter of more than academic or detached scientific importance. Except in situations of advanced or fulminating disease which would make the procedure excessively hazardous, resort to lung biopsy seems not only justified but clearly indicated in suspected instances of diffuse interstitial pulmonary fibrosis of this type. A personal experience in the past 5 or 6 years with at least 6 cases, of which 3 only have been here presented as the most characteristic and as representing both the extremes and the relative norm of clinical progression, suggests that the condition is one of relative and perhaps growing frequency.

In conclusion, I should like to quote the authors of the original description as they wrote in the summary of their 1944 report[7]. "This report describes a clinical and pathological entity characterized by a peculiar, progressive, diffuse fibrosis of the pulmonary alveolar walls, leading to deficient aeration of the blood with resulting dyspnea and cyanosis, and to enlargement and eventual failure of the right side of the heart. The etiology of the condition is at present undetermined." Little has been added since then to our knowledge of the cause, or causes. The passage of 25 years, however, has confirmed the essential accuracy of the original characterization of the underlying morbid process, as it has also the original conclusion that variations in the clinical picture depend on the stage this process has reached at the time the patients come under observation.

Summary. Three cases of diffuse interstitial fibrosis of the lungs are reported. The diagnosis in one, which was clinically of the fulminating type, was confirmed pathologically at postmortem examination. The diagnosis in the other 2, which were of the more chronic variety, was made by lung biopsy. The histologic features in all 3 resembled those first described by Hamman and Rich 25 years ago. Adrenocorticosteroid hormone therapy was administered to each of the 3 patients but produced no clearly beneficial effects in any. Neither was there evidence of adverse effects, either during the course of this therapy or upon its termination.

Two of the patients have died, one after only a month of severe symptoms, the other after 6 years of observation and treatment. The third patient has been observed over a period of 4 years. Though initially progressive, the disease has become apparently stabilized in this patient during the past 2 years. Despite the diversity in the clinical features it is believed that these 3 cases represent instances of the same underlying morbid process, which was originally characterized by Hamman and Rich.

The disease is no longer to be regarded as an extreme rarity and it can, in its most characteristic form, be differentiated from diffuse pulmonary fibroses due to known causes or those associated with systemic diseases, such as those of the collagen or rheumatoid group. Little is known of the etiology and pathogenesis of this disorder, and the treatment has, except in rare in-

stances, been reported unsuccessful. Nevertheless the diagnosis is of practical importance because of special problems which arise in the clinical management. Lung biopsy is indicated in suspected cases of diffuse interstitial fibrosis of this type, except in instances of advanced or fulminating disease which would make the procedure excessively hazardous.

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SUMMARIO IN INTERLINGUA

Observationes Concernente le Morbo de Hamman-Rich

Es reportate 3 casos de diffuse fibrosis interstitial del pulmones. In un—clinicamente del typo fulminante—le diagnose esseva confirmate pathologicamente per le necropsia. In le altere 2—ambes del varietate plus tosto chronic—le diagnose esseva establite per biopsia pulmonar. In omne le 3, le aspectos histologic esseva simile a illos primo describite 25 annos retro per Hamman e Rich. Therapia a hormones adrenocorticosteroide esseva usate in omne le 3 patientes sed produceva nulle effectos clarmente benefic in ulle de illes. Tamen, evidentia de effectos adverse esseva etiam absente, tanto durante le curso del therapia como post su termination.

Duo del 3 patientes ha morite, un post solmente un mense de sever symptomas, le altere post 6 annos de observation e tractamento. Le tertie patiente es sub observation depost 4 annos. In iste caso, le morbo esseva initialmente progressive, sed in le curso del passate 2 annos, illo se pare haber stabilisate. In despecto del diversitate in le aspectos clinic, il es opinate que iste tres casos representa casos del mesme subjacente processo morbide que esseva characterisate originalmente per Hamman e Rich.

Il es non plus possibile reguardar le morbo como un entitate de raritate extreme, e in su forma le plus characteristic illo pote esser differentiate ab diffuse fibroses pulmonar que ha un causa cognoscite o que es associate con morbos systemic, como—per exemplo—le morbos del gruppo collagenic o rheumatoide. Pauco es cognoscite con respecto al ethiologia e al pathogenese de iste disordine, e le tractamento—con rar exceptiones—ha essite reportate como insuccessose. Nonobstante, le diagnose es de importantia practic a cause del problemas special que se subleva in le tractamento clinic. Biopsias pulmonar es indicate in casos de suspicion de diffuse fibrosis interstitial de iste typo, excepte in patientes con le morbo in forma avantiate o fulminante, proque in tales le manovra de biopsia pulmonar esserea excessivemente hasardose.

ESOPHAGOSCOPY BY AND FOR THE INTERNIST: A REVIEW OF RESULTS IN A THOUSAND PATIENTS

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This paper presents a review of esophagoscopy performed in a thousand patients in the past 8 years with an esophagoscope using a flexible obturator for passage. These esophagoscopies were performed on the medical service of two hospitals. This is somewhat unusual since in many institutions esophagoscopy is not done by an internist but, depending on the local situation, remains in the province of the otolaryngologist or the thoracic surgeon. However, it is the internist who deals primarily with the diagnosis and treatment of most esophageal disease. Furthermore, endoscopy of the stomach is largely performed by internists who have a special interest in the gastrointestinal tract. The reason for this difference apparently stems from the fact that the type of esophagoscopy that was done up until recent years was with the rigid esophagoscope. As is well known, its development by the specialists in otolaryngology led to its being performed primarily by those specialists. However, with the advent of an esophagoscope that uses palpatory introduction in a very similar manner to the gastroscope, with reference to its passage through the hypopharynx, utilization of esophagoscopy by the internist began. A review of such diagnostic esophagoscopes was made by Schindler[6] who pointed out the ease and safety of esophagoscopy done in a similar manner to gastroscopy.

The Eder-Hufford esophagoscope has been used in this study but there are other similar makes that can be utilized. Its safety is attested by the fact that there have been only two perforations in this series of cases, and no fatalities. No adverse reactions from the premedication given in preparation for this procedure have been experienced. A further reason for this review is the fact that there are not many centers giving training to internists in this type of esophagoscopy. It is the belief of the author that esophageal disease is much more frequently found in institutions where esophagoscopy is considered a routine procedure rather than a procedure requiring elaborate preparation. The procedure can be performed very adequately as an outpatient procedure and approximately 25% of the patients reported herein were conducted on that basis. Repetition of the procedure in the same patient is also informative in some instances, such as results of surgery for portal hypertension in following the progress of esophageal varices. Repetition in cases of esophagitis may also be quite helpful, particularly in associated hiatal hernia, where the problems of ulcer and stricture may arise. In this group of patients 162, or 16.2%, had more than one esophagoscopic study. It is indeed unfortunate that many physicians who are not familiar with the manner in which the procedure is per-

formed have a rather distorted viewpoint about its dangers and discomfort which attitude is mirrored in the comments and fears of many of the patients prior to endoscopy.

Another aspect of what the author has learned with reference to esophagoscopy and assessment of the results is the comparison with the diagnostic accuracy of radiological study. Brick and Palmer[2] have reported on the comparison of diagnosis of esophageal varices by esophagoscopy and radiological study. It has been shown, and this has been confirmed by other reports (Katz, Friedman and Selesnick[3]), that the roentgenographic diagnosis of esophageal varices is not reliable. The same can be said of the diagnosis of esophagitis which is a difficult entity to establish on the basis of Roentgen-ray alone. The great value of roentgenography in diagnosis is not being underestimated but, as most radiologists will admit, there are certain areas in which such diagnostic accuracy can be improved. The use of radiological diagnosis combined with endoscopic diagnosis in the esophagus is one such area in which the combined procedures certainly does improve accuracy. It is for these reasons, among others, that a plea is made for wider education with reference to this endoscopic procedure in the hope that more physicians vitally interested in diseases of the esophagus will also become their own endoscopists.

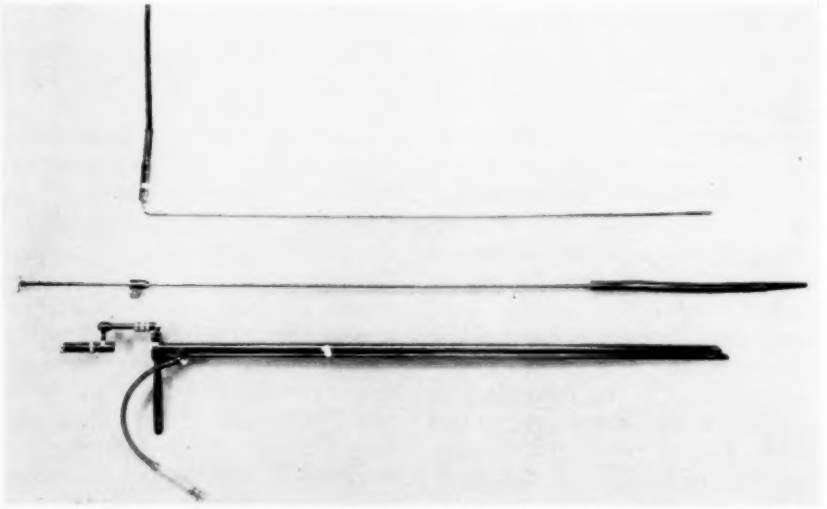

Fig. 1.—Eder-Hufford Esophagoscope. The flexible metal tip of the obturator with a rubber finger is shown. Note the telescopic lens giving four-diameter magnification and the channel for suction.

Method. The Eder-Hufford instrument that was used throughout this study is 51 cm. long, has a flexible obturator, and telescopic magnification of 4 diameters (Fig. 1). We have been impressed over the years by the ease in which this instrument can be passed by the palpatory technique. Admittedly, passage into the esophagus is a blind procedure. Once the esophagoscope is admitted into the esophagus, the remainder of the passage is done visually and essentially the same view is obtained as with orthodox rigid esophagoscopy, except that the telescopic magnification used gives clearer visual detail. After ade-

quate training with the instrument and demonstration of the actual procedure it has been my experience that senior residents and fellows in gastroenterology are quite capable of performing the procedure safely. In this particular study about 250 of the procedures were performed by trainees in my presence and under my supervision. It has been helpful, with beginners, to utilize edentulous patients for the initial esophagoscopies since passage of the esophagoscope is simple. This not only teaches the student the technique but gives him great confidence with reference to the ease in which the procedure can be performed.

Premedication in these cases has consisted of the use of Nembutal, 0.2 gm. orally 2 hours prior to the procedure, after an overnight fast. The ends of the capsules are perforated for quicker absorption. At the same time, 20 mg. of prochlorperazine intramuscularly is given. Additionally, 0.4 mg. of atropine sulphate is given subcutaneously one hour prior to the procedure. We have used 5 ml. of 2% Pontocaine as a gargle. We do not spray this medication nor introduce it into the esophagus. In the past 2 years it has been found that 100 mg. of meperidine hydrochloride given intravenously 5 minutes prior to endoscopy affords very good relaxation and cooperation by the patient, and this method is more frequently being used, especially with outpatients. On the average, in 3 hours, most patients are free of the effects of these types of analgesia and sedation.

The position of the patient is in the left lateral decubitus with his knees flexed into his abdomen. The nurse holds the head in the position directed by the examiner. The procedure proceeds very much in the same manner that gastroscopy does. An advantage of esophagoscopy over the usual type of gastroscopy is that suction can be utilized and biopsies can be directly taken from suspicious lesions. In this series biopsy material was obtained in 84 cases, without untoward result in any.

INDICATIONS. The reasons for esophagoscopy in the 1000 patients are listed in Table 1. In addition to the usual indications for esophagoscopy, namely dysphagia or any difficulty with the swallowing mechanism, every patient with chronic liver disease, primarily cirrhosis, is esophagoscoped to determine the presence of varices. This is part of the routine study of any patient with chronic liver disease. No distinction is made between patients who have had gastrointestinal bleeding and those who have not inasmuch as the information of the presence of varices may be an important aspect in the future management of the patient with chronic liver disease. It is for this reason that there is a large number of cirrhotic patients included. The lack of reliability of the diagnosis of esophageal varices by Roentgen-ray, particularly when the varices are small to moderate in size, has made esophagoscopy an integral part of the study of such patients. Comparison of the diagnosis of such varices, in 300 biopsy-proved cases of cirrhosis, with and without a history of gastrointestinal bleeding, with Roentgen-ray study, is noted in Tables 2, 3, 4.

TABLE 1.—INDICATIONS FOR ESOPHAGOSCOPIES IN 1000 PATIENTS

Cirrhosis or Chronic Liver Disease	414
Roentgen-ray Lesion Diagnosed	
1. Hiatus Hernia	125
2. Filling Defect (cancer or stricture)	57
3. Megaesophagus	21
4. Esophagitis or Ulcer, or both	15
5. Varices	14
Dysphagia or Esophageal Symptoms (pain) (negative Roentgen-ray)	74
Upper G.I. Bleeding	
Emergency or Active	169
Routine	60
Esophagitis (suspected clinically)	32
Organic Foreign Bodies	8
Post-antibiotic Dysphagia	6
Scleroderma	5

Another reason for esophagoscopy is the finding by Roentgen-ray of an esophageal lesion. In Table 1, it is noted that hiatal hernia is not an infrequent reason for esophagoscopy. Many times, in addition to the hiatus hernia, which

sometimes is diagnosed by esophagoscopy when the roentgenographic report is negative (Palmer[4]), esophagitis, which accompanies the hiatus hernia, is found. The clinical impression of the presence of esophagitis because of pain on swallowing or with ingestion of food, together with pain in the substernal area, has also been a reason to esophagoscope some patients.

TABLE 2.—INCIDENCE OF ESOPHAGEAL VARICES DETERMINED ESOPHAGOSCOPICALLY IN CIRRHOSIS

Total Cases	300
Esophageal Varices	190
Esophageal Varices Plus Hemorrhage	94
Esophageal Varices and no Hemorrhage	96

TABLE 3.—INCIDENCE OF ESOPHAGEAL VARICES CORRELATED WITH HISTORY OF HEMORRHAGE

Cases		300
History of Hemorrhage		118
Varices	94	
No History of Hemorrhage		182
Varices	96	

TABLE 4.—COMPARISON OF ROENTGEN-RAY AND ESOPHAGOSCOPY IN DIAGNOSIS OF ESOPHAGEAL VARICES IN 300 BIOPSY-PROVED CASES OF CIRRHOSIS

		Roentgen-Ray	
Esophagoscopy		*Varices*	*No Varices*
Varices	190	44	146
No Varices	110	9	101

TABLE 5.—RESULTS OF RADIOLOGICAL AND ESOPHAGOSCOPIC EXAMINATIONS (Modified from Spiro, M.: Brit. J. Radiology, **31**, 626, 1958.)

Positive Findings By	*Esophagitis*	*Ulcer*	*Stenosis*
Roentgen-ray	0	2	4
Roentgen-ray and Esophagoscopy	12	7	23
Esophagoscopy only	53	5	0
Totals	65	14	27

The diagnosis of esophagitis by Roentgen-ray is made infrequently and dependence upon radiological diagnosis as the sole diagnostic technique overlooks an important aspect of esophageal disease since this disease does occur more frequently than is noted by Roentgen-ray. On this particular point, it is interesting that Spiro[8] recently indicated in 65 cases of esophagitis that the roentgenographic diagnosis alone was made in no case and was made esophagoscopically alone in 53 of the 64 patients. Table 5 is taken from this article[8] and indicates the comparison between the radiologic and esophagoscopic diagnoses in esophagitis.

Another area in which esophagoscopy is often helpful and certainly reassuring is in patients in whom esophageal symptoms, such as difficulty in swallowing or pain in the substernal or xiphoid area, exist with a perfectly normal roentgenogram. A large proportion of these patients may have functional esophageal disease. Ruling out organic disease is extremely important in the management of this group of patients. In some of these patients esophageal disease is noted.

The author[1] has indicated the desirability of esophagoscopy in the diagnosis and management of massive upper gastrointestinal bleeding. It is interesting to note that 169 patients underwent esophagoscopy because of massive bleeding on an emergency basis, or while active bleeding persisted, to determine whether a lesion could be found. The great value of the negative findings, particularly with reference to esophageal varices, was pointed out previously (Brick[1]) and has proved to be very helpful in de-

termining the necessity for emergency subtotal gastrectomy for upper gastrointestinal bleeding in which the patient's condition is deteriorating. In the routine case of upper gastrointestinal bleeding, in which the patient after medical treatment usually stops bleeding in the hospital, esophagoscopy is done less frequently because roentgenographic diagnosis often determines the site of bleeding. This is not true in my experience in the massively bleeding patient inasmuch as the presence of blood in the stomach and esophagus, as well as the condition of the patient, often makes it difficult for an adequate examination. Roentgenographic examination under these conditions may also give rise to false positive results because of blood clots in the stomach and duodenum and false negative results, particularly in gastric and duodenal ulcer, because of blood clots in the ulcerated area.

COMPLICATIONS. In this series there have been complications in 2 cases, both perforation of the esophagus. Case 1 was a 52-year-old Indian man who had biopsy-proved cirrhosis and experienced an episode of gastrointestinal hemorrhage 2 weeks previously. Roentgenographic study of the esophagus was reported to be negative. Esophagoscopy revealed moderately large esophageal varices involving the lower half of the esophagus. He experienced no pain during or after the procedure but 4 hours later complained of pain over the upper chest. However, at that time there was no emphysema of the tissues of the chest and neck. The next morning the patient felt well enough to eat breakfast but during this meal experienced severe pain over the upper chest. Examination showed emphsema in the tissues of the neck on the left side. This was confirmed by a roentgenogram of this area but no air was seen in the mediastinum. A mediastinal exploration was done the same day and no infection was found. The area was drained and 5 days after surgery the patient was able to eat and experienced no difficulty thereafter.

Case 2 was a 43-year-old woman whose main complaint was difficulty in swallowing and pain in the mid-chest of 2-weeks' duration. Roentgenographic study was negative. The patient was known to be quite nervous and a diagnosis of chronic anxiety state had been made. However, because of her fear that she had cancer, her physician requested esophagoscopy. The procedure was done without any difficulty but immediately after the esophagoscope was withdrawn the patient complained of severe pain in the lower left chest. consistent with pneumothorax which was confirmed by a roentgenogram of the chest. Surgical exploration was performed within 2 hours of the completion of esophagoscopy and a perforation 1.2 cm. long was demonstrated 4 cm. above the cardioesophageal junction. No evidence of accompanying inflammation was noted and the esophageal perforation was sutured. The patient had an uneventful postoperative course and was discharged 8 days after surgery. Her subsequent course, with reference to the esophageal perforation, has been completely uneventful.

After esophagoscopy, the patient may experience soreness in the throat or neck which usually disappeared within 24 to 48 hours. The use of soothing lozenges or saline gargles has been quite helpful for the sore throat.

Many physicians have asked the author about the danger of bleeding resulting from esophagoscopy either in a patient who has gastrointestinal hemorrhage with the site in the esophagus or from esophageal varices that are not bleeding. Bleeding after esophagoscopy has not been encountered in this group of patients. Palmer and Wirts[5] reported

no bleeding episodes in a survey of 40,540 esophagoscopies performed by 119 doctors.

This review indicates the safety and the value of esophagoscopy. In my opinion the use of esophagoscopes with a flexible obturator, passed in the same manner as the gastroscope, has made feasible the availability of this procedure for the internist interested in gastrointestinal diseases. Two studies (Selesnick and White[7], Stempien *et al.*[9]) reported a total of 297 esophagoscopic examinations using a similar type of esophagoscope than the one used in this study but of a different manufacture. In none of these cases was there a complication. A study comparable to the present one was reported recently by Sullivan and Myers[10] who used the same type of esophagoscope as that used in the present review. In their study 860 esophagoscopies in 661 patients were reported with 3 perforations, two hypopharyngeal and one mid-esophageal. Only one of these patients required surgery and there were no deaths. It is interesting that treatment of hypopharyngeal perforations can be carried out successfully by the use of antibiotics and interdiction of oral feedings for several days. The indications for esophagoscopy given by these authors is quite similar to those in the present study.

Summary and Conclusions. 1. Results of esophagoscopy performed in 1000 patients with an esophagoscope using a flexible obturator and palpatory introduction are presented.

2. Two perforations with no mortality, both treated surgically, were the complications encountered.

3. The practicability and diagnostic usefulness of this method of endoscopy for the internist is described.

4. The diagnosis of esophageal varices and esophagitis by esophagoscopy is compared with the diagnosis by radiological techniques.

5. It is believed that every patient with chronic liver disease should have esophagoscopy to determine the presence of esophageal varices. A much higher incidence of such varices exist in these patients, even in the absence of gastrointestinal hemorrhage, than can be delineated by diagnostic radiological procedures.

6. It is believed that sufficient experience has been obtained by enough workers in this field to indicate the safety and value of the procedure and the necessity for a broader teaching program to train more internists with gastrointestinal interests in this technique.

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SUMMARIO IN INTERLINGUA

Esophagoscopia per e pro le Internista: Un Revista del Resultatos in Mille Patientes

1. Es presentate le resultatos del esophagoscopias effectuate in 1000 patientes per medio de un esophagoscopio usante un obturator flexibile e un technica de introduction palpabile.

2. Duo perforationes, ambes tractate chirurgicamente, esseva le sol complicationes incontrate. Le mortalitate esseva zero.

3. Es describite le practicabilitate e le utilitate diagnostic de iste methodo de endoscopia pro le internista.

4. Le diagnose esophagoscopic de varices esophagee e de esophagitis es comparate con le diagnose per medio de technicas radiologic.

5. Es opinate que omne patiente con chronic morbo hepatic deberea esser subjicite a un studio esophagoscopic pro determinar le presentia o absentia de varices esophagee. Un grandemente augmentate incidentia de tal varices existe in iste patientes, mesmo in le absentia de hemorrhagia gastrointestinal, in comparation con lo que pote esser delineate per methodos de diagnose radiologic.

6. Es opinate que un sufficiente grado expertessa ha essite attingite per un sufficiente numero de travaliatores in iste campo pro justificar le assertion que le methodo es salve e utile e que il existe le urgente desiderato de un plus extense programma de instruction pro trainar plus internistas con interesses gastrointestinal in le correspondente technicas.

SUMMARIO IN INTERLINGUA

(See page 329 for original article)

Hemoglobinas Anormal.
II. Le Pathologia del Character de Cellula Falciforme

Le assecurate differentiation del character de cellula falciforme ab morbo de hemoglobina S-C, S-thalassemia, e mesmo anemia a cellula falciforme es possibile solmente super le base de meticulose studios hematologic e electrophoretic. Isto vale etiam in le presentia de datos necroptic. Le difficultate es particularmente pronunciate in patientes de etate juvenil.

Le presentia ante morte de definite infarctos visceral in 22 de 135 patientes con provate character de cellula falciforme signala le periculos que inhere in iste morbo vaso-occlusive. Le ben-documentate occurrentia de hematuria e infarcimento splenic durante volos a grande altitudes in casos de character de cellula falciforme augmenta le importantia del resultatos del presente studio necroptic.

Le character de cellula falciforme pote devenir e de facto deveni le causa de morte in e per se ipse, sed in su rolo letal illo es associate plus frequentemente con altere morbos o statos anoxemic, como per exemplo intoxication alcoholic, anesthesia chirurgic, e insufficientia cardiac. Pro iste ration, le scrutinio de omne patientes negre es recommendate, insimul con le effectuation de mesuras a prevenir anoxia in individuos con morbo de cellula falciforme.

COMPARATIVE TOXICOLOGY OF IRON COMPOUNDS

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The great number of iron preparations available for the treatment of iron deficiency anemia is evidence of the incidence and seriousness of the clinical problem and the lack of a universally acceptable preparation for therapy. The principal problems accompanying oral administration of iron preparations involve gastrointestinal distress and, more importantly, serious and even fatal iron toxicities, especially in children.

This report is concerned with the animal toxicities of a new high molecular iron-carbohydrate complex‡ compared to those of several other preparations.

Materials and Methods. Iron-carbohydrate complex represents a ring structure in which the metallic (Fe) ion is sequestered and firmly bound within a polymerized carbohydrate of high molecular weight (30,000). In physical properties, it is a free-flowing, amorphous, brown powder highly soluble in water and is exceptionally high in iron content (approximately 50%). It forms a dark brown colloidal solution that is stable at pH 4 to 11 and to heat.

Other compounds used were exsiccated ferrous sulfate (Fe 29.7%); ferrous gluconate (Fe 11.6%); ferrous fumarate§ (Fe 32.9%); ferric choline citrate (Fe 12.0%); an iron polysaccharide complex||, which contains 20 mg. of trivalent iron per milliliter (given by intravenous injection), and tablets of ferroglycine sulfate complex, available commercially as Ferronord, which were used in pulverized form.

Acute toxicity in mice. The compounds were administered as aqueous solutions where possible, otherwise as fine suspensions. Groups of 10 or more male albino Swiss-Webster mice were given the compounds (Table 1) intravenously (i.v.), intraperitoneally (i.p.), or intragastrically (i.g.). The rate of i.v. injections was 0.01 ml. per second. The animals were observed closely for several hours following injection, and the LD_{50} and 95% confidence limits were determined at the end of 24 hours by the method of Litchfield and Wilcoxon[5]. The animals receiving iron-carbohydrate complex and ferrous sulfate were observed for a period of 7 days following injection and any delayed manifestations of toxicity were recorded. If any deaths occurred after 24 hours, the LD_{50} was recalcu-

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||Kindly supplied by Dr. A. P. Truant, Astra Pharmaceutical Products, Inc., Worcester, Mass.

TABLE 1.—ACUTE TOXICITIES OF IRON COMPOUNDS IN MICE

		LD_{50} (19-20 Confidence Limits), mg./kg.							
		i.v. route			*i.p. route*			*i.g. route*	
Compound	*% Fe*	*No. mice*	*As salt*	*As Fe*	*No. mice*	*As salt*	*As Fe*	*No. mice*	*As salt*	*As Fe*
Iron-carbohydrate complex	48.8	40	175 (158–194)	85	30	980 (834–1151)	478.2	30	>8000	>3904
Ferrous sulfate	29.72	55	112 (105–120)	33	105	137 (122–154)	40.7	40	1025 (802–1311)	305
Ferrous gluconate	11.58	55	199 (182–218)	23	30	160 (94–272)	18.5	100	3950 (3543–4404)	457.4
Ferrous fumarate	32.87	—	—	—	40	480 (410–562)	157.8	70	1570 (1353–1821)	516.1
Ferroglycine sulfate complex	15.87	—	—	—	80	365 (341–391)	57.9	50	1940 (1516–2483)	307.9
Ferric choline citrate	12.02	70	210 (192–229)	25	40	151 (120–190)	18.1	40	5500 (4297–7040)	661.1
Iron polysaccharide complex	2.0	30	—	170 (147–197)	50	—	318 (238–426)	—	—	—

lated at the end of the 7-day observation period. Strain and sex differences in response to iron-carbohydrate complex and ferrous sulfate were evaluated by the use of female albino Swiss-Webster, black female BDF-1 and C-57 and female fawn DBA-2 mice.

ACUTE TOXICITY IN RATS. Male (250 to 350 gm.) and female (150 to 250 gm.) Harlan-Wistar rats in mixed groups of 6 received the iron compounds i.g. in attempts to determine 24-hour LD_{50} in this species.

TOXICITY IN DOGS. Mongrel dogs, unselected as to sex, were used in all acute toxicity studies. Acute, rapid, i.v. injections were made and the LD_{50} determined at 24 hours. In subacute toxicity tests, dogs received iron-carbohydrate complex or ferrous sulfate in gelatin capsules twice daily for approximately one month. The total dose of ferrous sulfate was 0.5 gm. per day (5 dogs) and the total daily doses of iron-carbohydrate complex were 0.5 gm. (3 dogs), 1.0 gm. (3 dogs), and 2.0 gm. (6 dogs). These animals were observed closely for emesis and other outward signs of toxicity. At the end of the test period the dogs were killed (pentobarbital sodium solution intracardially and exsanguination) and subjected to extensive histopathologic studies.

PATHOLOGIC STUDIES. Blood samples from all dogs used in subacute toxicity tests were taken for routine hematologic studies, and thorough necropsy examinations were made for gross lesions.

Fresh specimens of liver, spleen, bone marrow, stomach, small intestine, large intestine, brain, kidneys, adrenal glands, mesenteric lymph nodes, thyroid, and myocardium were fixed in formalin, processed, and sections (about 6 μ) of all tissues were stained with azure-eosin. In addition, sections of liver, spleen, and bone marrow were stained by a modified Gomori's method for iron.

EMETIC STUDIES. The various iron compounds were given in suspension (ferrous sulfate), in solution (iron-carbohydrate complex), or in gelatin capsules. The dogs were not fasted, and no dog was used more than once in these studies.

Results. The results in mice are summarized in Table 1. Since the iron content of the compounds varies considerably comparisons were made on the basis of actual iron content. The iron polysaccharide complex was the least toxic by the i.v. route in mice and iron-carbohydrate complex was next. The other compounds tested were 2 to 4 times as toxic as the iron-carbohydrate complex. The iron-carbohydrate complex was the least toxic of the compounds studied i.p. in mice, though not significantly less toxic than the iron polysaccharide complex. It had only 1/10 the toxicity of ferrous sulfate. Intragastrically, iron-carbohydrate complex was at least 6 times less toxic than any of the other compounds tested. The volumes necessary made it impractical to attempt to determine the i.g. toxicity of the iron polysaccharide complex. There was no significant difference between the 1- and 7-day toxicities for either iron-carbohydrate complex or ferrous sulfate for the i.v. and i.g. routes in mice.

In Swiss-Webster male mice the i.g. LD_{50} was 1025 mg. per kg. for ferrous sulfate. The same dose in female mice of different strains (10 mice in each test) produced the following percentages of deaths: Swiss-Webster, 70; BDF-1, 70; and C-57, 90. The i.g. LD_{50} of iron-carbohydrate complex in male Swiss-Webster was >8000 mg. per kg. The same dose produced no deaths in female Swiss-Webster, BDF-1, C-57 and DBA-2. Thus, there is no evidence of sex or strain differences in these very limited studies.

Results of acute toxicity studies in rats and dogs are summarized in Table 2. None of the compounds are very toxic following i.g. administration to rats and evidently there are only slight differences in the lethal effects of ferrous sulfate, ferrous gluconate and ferroglycine sulfate complex. There was no significant difference between the 1- and 7-day LD_{50} for ferrous sulfate and iron-carbohydrate complex in rats.

Following i.v. administration in dogs, ferric choline citrate and ferrous sulfate were the most toxic of the compounds tested. Ferrous gluconate and

TABLE 2.—ACUTE TOXICITIES OF IRON COMPOUNDS IN RATS AND DOGS

			LD_{50} (19/20 Confidence Limits)				
			i.g. route			*i.v. route*	
Compound	*% Fe*	*No. rats*	*As salt*	*As Fe*	*No. dogs*	*As salt*	*As Fe*
Iron-carbohydrate complex	48.8	10	>8000	>3904	18	94 (78–113)	45.9
Ferrous sulfate	29.7	24	2625 (2323–2966)	780	16	79 (71–88)	23.5
Ferrous gluconate	11.6	24	7460 (6844–8131)	865	9	>400	>46.4
Ferrous fumarate	32.9	24	>7080	>2329	—	—	—
Ferroglycine sulfate complex	15.9	24	5590 (4454–7014)	894	—	—	—
Ferric choline citrate	12.0	12	>8000	960	17	140 (101–190)	16.8
Iron polysaccharide complex	2.0	—	—	—	3	—	>40

iron-carbohydrate complex were the least toxic. The iron polysaccharide complex was given in a dose of 40 mg. per kg. to 3 dogs without lethal effects. Because of the fixed concentration of the solution, the volume necessary for higher dosages was too great to be practical.

weights of 6 dogs on the 2 gm. per day dose of iron-carbohydrate complex showed a 0.1 kg. rise as the maximum change. Emesis occurred once in the 12 dogs during the study with iron-carbohydrate complex and this was at the dose of 0.5 gm. per day. In contrast, emesis occurred 14 times during

TABLE 3.—EMETIC EFFECTS OF IRON COMPOUNDS IN DOGS

Compound	*Form*	*Dose mg./kg.*		*No. Vomiting/ Total No.*	*% Emesis*
		As salt	*As Fe*		
Ferrous sulfate	Suspension	96	28.8 (19.5–42.6)	—	ED_{50}*
	Capsule	62	18.6 (15.3–22.8)	—	ED_{50}
Iron-carbohydrate complex	Solution	600	293	0/4	0
		900	439	0/7	0
	Capsule	300	146	0/2	0
		600	293	1/4	25
		900	439	0/10	0
Ferrous gluconate	Capsule	400	46	2/5	40
		800	93	5/5	100
Ferrous fumarate	Capsule	800	263	4/6	67
Ferroglycine sulfate complex	Capsule	200	32	2/4	50
		400	64	2/2	100
		800	127	2/2	100
Ferric choline citrate	Capsule	800	96	1/5	20
		1200	144	2/6	33

*ED_{50} = Emetic dose for 50% of dogs.

Emetic responses to the various iron compounds in dogs are presented in Table 3. Iron-carbohydrate complex produced less gastrointestinal distress as indicated by emesis than any of the other compounds. Ferrous sulfate and ferroglycine sulfate complex were the most emetic in this study.

In the subacute toxicity studies average weights of dogs given ferrous sulfate and those given iron-carbohydrate complex at the two lower dosage levels decreased the first week and then remained constant or were regained. The changes ranged between 0.6 and 0.8 kg. for the 3 groups. The average

the same period in the 5 dogs given ferrous sulfate. No other gross signs of toxicity were observed.

No gross lesions or microscopic lesions suggestive of iron toxicity were observed in any of the dogs regardless of the compound or dosage level. No significant differences in stained iron content were apparent in the spleen, liver, and bone marrow at any dosage level of either ferrous sulfate or iron-carbohydrate complex. The total red blood cell count and hemoglobin levels were within the normal range in each dog.

Discussion. Studies of ferrous sulfate,

ferrous gluconate and ferrous fumarate in rodents gave results comparable to those reported by other investigators (Table 4). The consistently lower toxicity observed in our studies for these compounds in rats might be explained by the fact that our studies were done in older animals that had free access to food except during the period of testing; no sex differences were observed in these studies or in the studies conducted in mice.

stained iron content in the spleen, liver, or bone marrow from lower dosages or from ferrous sulfate. This suggests that even high doses of iron-carbohydrate complex may be tolerated without serious side effects.

Summary. 1. Studies in mice indicate that iron-carbohydrate complex is less toxic than ferrous sulfate, ferrous gluconate, ferrous fumarate, ferroglycine sulfate complex, ferric choline citrate and iron polysaccharide complex by the oral and intraperitoneal routes. The iron polysaccharide complex by the intravenous route was the least toxic and iron-carbohydrate complex was next least toxic.

2. None of the compounds tested was less toxic than iron-carbohydrate complex by the oral route in rats or the intravenous route in dogs.

3. Iron-carbohydrate complex produced the least gastrointestinal irritation as indicated by emesis in the dog.

4. Doses of iron-carbohydrate complex equivalent to 1 gm. elemental iron per day for a month failed to produce local irritation or systemic alterations in dogs.

TABLE 4.—ACUTE TOXICITIES OF IRON COMPOUNDS IN MICE AND RATS

	LD_{50}, *mg. Fe/kg.*			
Compound	*mice, i.g.*	*mice i.v.*	*rats, i.g.*	*Reference*
Ferrous sulfate	305	33	780	
	306	13	298	Hoppe *et al.*[3]
	230	11	344	Berenbaum *et al.*[1]
				Nissim[6]
				Keith[4]
	900	14		Somers[7]
				Edge *et al.*[2]
Ferrous gluconate	457	23	865	
	429	13	518	Hoppe *et al.*[3]
	320			Berenbaum *et al.*[1]
Ferrous fumarate	516		>2329	
	630		580	Berenbaum *et al.*[1]

Iron-carbohydrate complex showed a low order of toxicity in 3 species of laboratory animals. With one exception none of the 6 iron compounds tested was less toxic than iron-carbohydrate complex; the iron polysaccharide complex was less toxic by the i.v. route in mice. Further, gastric intolerance as indicated by emesis was very much less for iron-carbohydrate complex than for the other 5 compounds. In doses equivalent to 1 gm. of elemental iron daily for a month, gross or microscopic changes were not found in dogs. Large doses of iron-carbohydrate complex (2 gm. per day) produced no significant differences in

ACKNOWLEDGMENTS: The authors wish to thank Miss Betty Alvey, Miss Sallie Kimble and Mrs. Lilah Estill for technical assistance.

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SUMMARIO IN INTERLINGUA

Toxicologia Comparative de Compositos de Ferro

1. Studios in muses indica que—in administrationes per via oral e per via intraperitonee—complexo de ferro e hydrato de carbon es minus toxic que sulfato ferrose, gluconato ferrose, fumarato ferrose, complexo de ferroglycina e sulfato, ferric citrato de cholina, e complexo de ferro e polysaccharido. In administrationes per via intravenose, complexo de ferro e polysaccharido esseva le minus toxic, sequite per complexo de ferro a hydrato de carbon.

2. Nulle del compositos testate esseva minus toxic que complexo de ferro e hydrato de carbon per via oral in rattos o per via intravenose in canes.

3. Complexo de ferro e hydrato de carbon produceva le minus marcate grado de irritation gastrointestinal, a judicar per le emesis provocate in canes.

4. Doses de complexo de ferro e hydrato de carbon equivalente a 1 g de ferro elementic per die durante un mense non produceva irritation local o alterationes systemic in canes.

SUMMARIO IN INTERLINGUA

(See page 309 for original article)

Le Susceptibilitate de Bacillos de Species de Proteus e Providence pro 10 Agentes Antibacterial

Per medio de un meticulosemente standardisate methodo de dilution a tubos, 95 isolatos clinic de *Proteus* (representative del usual distribution de species in le Statos Unite) e 12 isolatos clinic de bacillos del gruppo Providence esseva testate relative a lor susceptibilitate pro 10 agentes anti-infectiose. Omne agente esseva testate a un numero de concentrationes. Tamen, le efficacia de omne le agentes individual esseva judicate a un concentration experimental correspondente al concentration sanguinee que es attingibile in le patientes.

Penicillina esseva le agente le plus efficace contra *Proteus mirabilis*. Kanamycina esseva le agente le plus efficace contra *P. rettgeri, P. morgani, P. vulgaris*, e bacillos del gruppo Providence.

A NEW METHOD OF MEASURING THE EFFECT OF DRUGS ON PERFORMANCE*

By Leo J. Cass, M.D.

PHYSICIAN, UNIVERSITY HEALTH SERVICES, HARVARD UNIVERSITY, CAMBRIDGE, MASSACHUSETTS; CHIEF OF MEDICINE, BROOKS HOSPITAL, BROOKLINE MASSACHUSETTS; AND VISITING PHYSICIAN, LONG ISLAND HOSPITAL, BOSTON, MASSACHUSETTS

AND

Willem S. Frederik, Ph.D., M.D.

LECTURER, HARVARD SCHOOL OF PUBLIC HEALTH, BOSTON, MASSACHUSETTS AND DIRECTOR OF RESEARCH, LIBERTY MUTUAL RESEARCH CENTER, HOPKINTON, MASSACHUSETTS

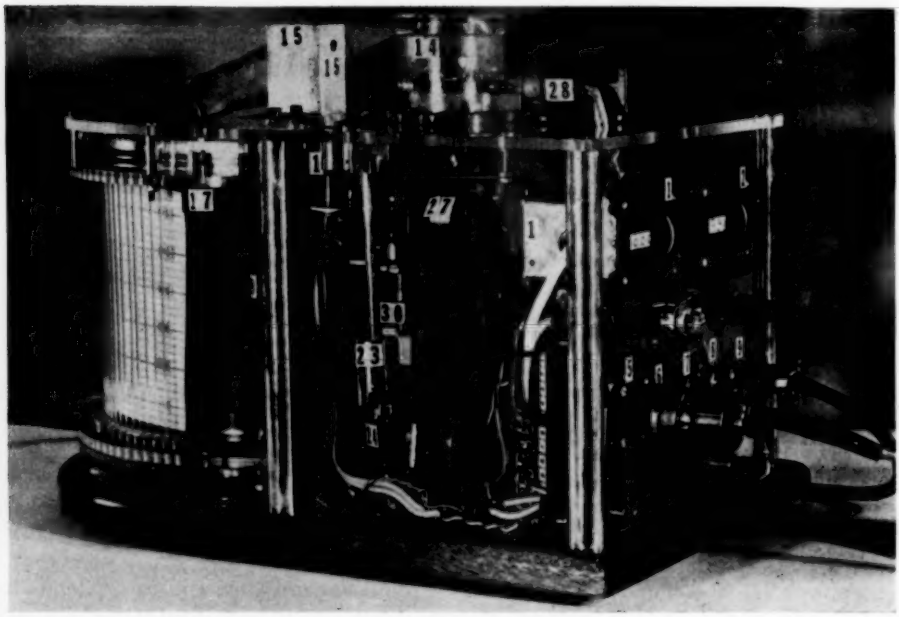
In spite of a number of methods and techniques for measuring the action of drugs in animals which have been reported, there remains a shortage of objective methods for measuring the effect of drugs on human performance. Nevertheless, we are constantly confronted by the fact that it is in humans that the final evaluation of any drug must be performed. While it is recognized that actual clinical trials must remain the ultimate proof of the usefulness of a drug, a search for objective procedures which might be applied to humans and which might further help to delineate and to clarify the area of effectiveness of a given drug seems to be eminently worthwhile. It is hoped that such an evaluation will yield information which will help establish in advance the potential usefulness of the drug.

One of the authors devised some years ago a method to evaluate so-called "objective fatigue" (Frederik[3]). This approach called for the accurate measurements of simple reaction times to stimuli such as flash of light, making a sound, and the like. It was found that the distribution of reaction times in a statistically significant number of trials was of particular interest. This finding made it desirable to develop further a specific instrument which would present the results of a series of reaction time measurements in the form of a distribution curve (Frederik[4]).

The Performance Indicator[2] lends itself to the measurements of the effect of drugs on reaction time in humans. The machine measures in this application a type of simple performance, to be precise, the response-time to the stimulus of lights turned on at varying intervals. The amount of time required by the subject to respond to the light is recorded by a single small ball dropping into transparent tubes set around the periphery of a cylinder at equal intervals. Thus, a visual record of the results in the form of a distribution curve is produced.

A series of 100 such individual tests causes a graph to be made up of the 100 pellets as they fall into the various tubes. These tubes are spaced so that they are separated by a distance equivalent to the travel time of 1/50th of a second. This interval represents the degree of sensitivity of the machine. A complete test consists of 100 trials per-

*From a scientific exhibition at the annual meeting of the American Medical Association, June, 1960, Miami, Florida.



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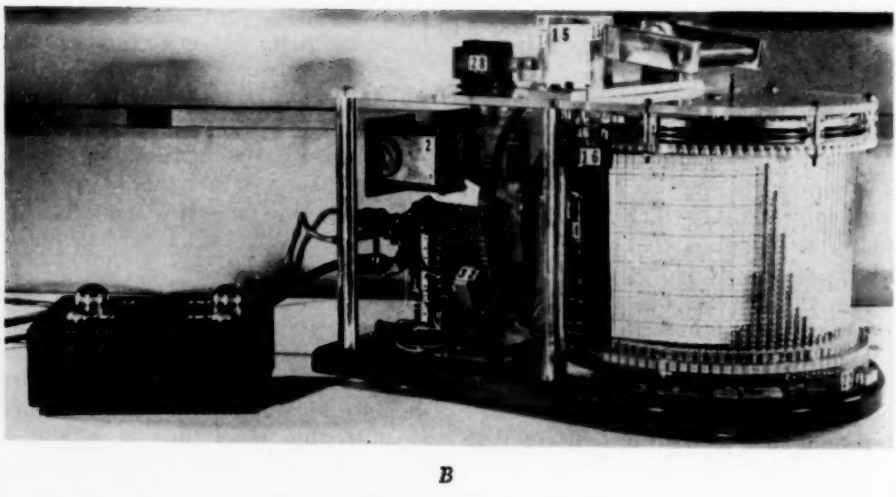

B

Fig. 1.—The Performance Indicator.

formed during approximately a 5½ minute period after which the machine automatically shuts itself off. Figure 1 shows the machine as assembled. The number of errors is also recorded by the machine for each set of tests. The device is so constructed that it is practically impossible to predict a stimulus and after 2 or 3 test runs, the response of the test subject is essentially constant. The machine tests the time required for an individual to perceive the stimulus, coordinate the muscle response, and respond by pressing a switch button. These actions are essentially those of the central nervous system and lend themselves to precise measurements by this machine. The changes in this time interval produced by drugs and the variation of the test subject's response can serve as a measure of the effect of a drug upon such reactions.

The first application of this machine in the field of drug research was to determine the effect of alcohol on performance. A group of volunteers was first given the test to establish a normal reaction curve as a control. They were then given 1½ ounces of whiskey (bourbon) in water every 30 minutes. Tests were performed at the start of the trial and consequently at the end of every 30 minute period. Figure 2 presents some of the results of this investigation. The first curve shows the result of the reaction time measurement before the intake of alcohol. The second graph is taken 1½ hours later showing the results on reaction times of 3 drinks. The last graph, taken 2½ hours after the beginning of the experiment, shows the results of 5 drinks of 1½ ounces of whiskey on the reaction time distribution. The number of errors in these tests were 1%, 3%, and 7%, respectively, which correlated well with the number of drinks. These graphs demonstrate that while alcohol does produce a prolongation in the average reaction time, this is not as striking as the change in the distribution curve itself. The curve is prolonged and flattened and there is a much greater incidence of very slow reactions. This series of tests serves to demonstrate the general potentialities of the machine and indicates some possible applications.

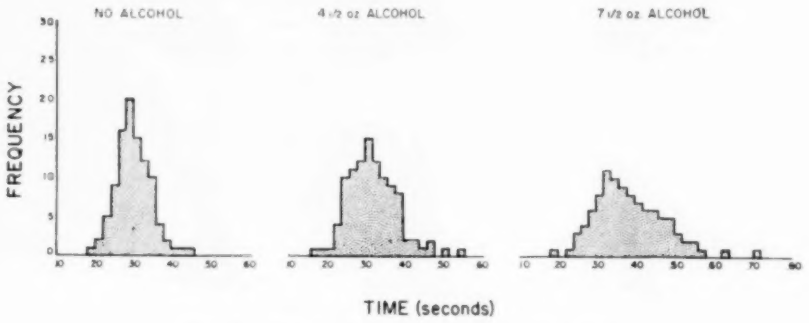

Fig. 2.—Effect of alcohol upon reaction time.

We have previously published the effect of several amphetamine and non-amphetamine preparations on appetite control (Cass[1]). With this in mind, a study was undertaken to determine whether or not such preparations would have any effect on the reaction time as tested by this method. If such an effect could be shown, it should also

be possible to measure how long this effect would last. This is of special importance to demonstrate the differences between long-acting and short-acting forms of drugs. Figure 3 shows the effect of 20 mg. doses of amphetamine as the phosphate and as the ion exchange resin complex* on reaction time as compared to placebo medication. Observations were made at ½, 4½ and 8½ hours after administration of the drug or the placebo. It will be noted that amphetamine tends to produce a small number of rapid responses, that is, 5 to 7% of the trials fall into a shorter time interval. The average reaction time may not be appreciably shortened. It appears that the reaction time returns to normal before 8½ hours after the soluble salt form of the drug is administered, whereas the effect is still demonstrable after 8½ hours when the same dose of the drug is administered as the ion exchange resin complex. Thus, this action is sustained in the latter drug.

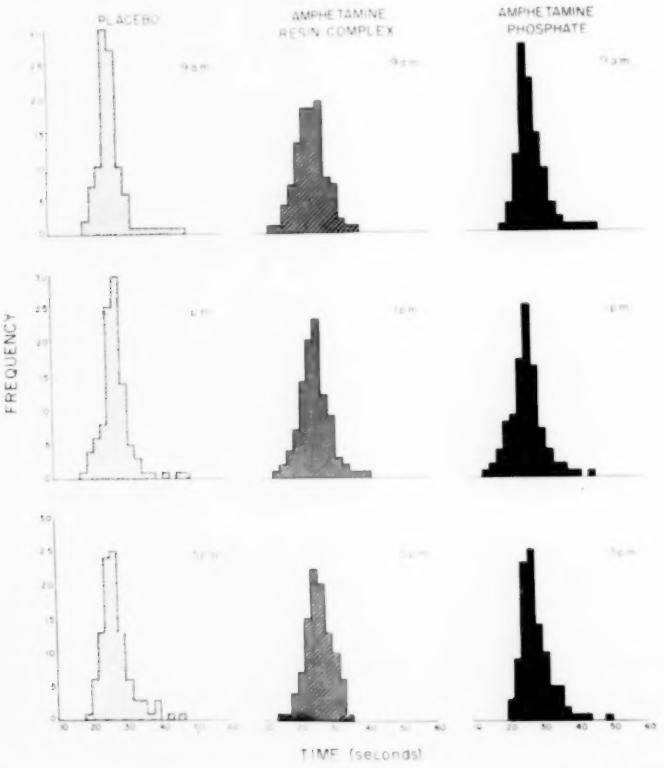

Fig. 3.—Effect of two forms of amphetamine upon reaction time in comparison with a placebo.

Figure 4 illustrates that the non-amphetamine drug, phentermine resin†, although reported to have an appetite suppressant activity equivalent to am-

*Biphetamine, supplied by the R. J. Strasenburgh Co., Rochester, New York.
†Ionamin, supplied by the R. J. Strasenburgh Co., Rochester, New York.

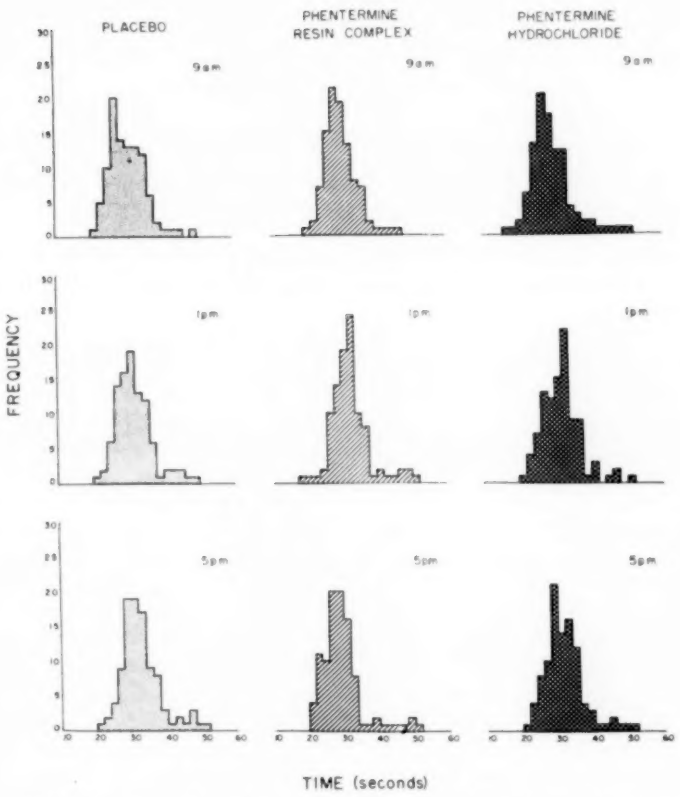

Fig. 4.—Negative effect of two forms of phentermine on reaction time in comparison with a placebo.

phetamine (Cass[1], LeRiche[5]), has no demonstrable effect on reaction time as measured by this device in comparison to a placebo.

Conclusions. It has been shown that a machine which measures reaction time quantitatively in humans can be used to study the effect of drugs on this function.

Alcohol taken as whiskey increases the reaction time, alters the performance curve and increases the number of errors. These effects can be measured quantitatively.

Data showing the effect and duration of effect of amphetamine as the soluble salt and as the ion exchange resin complex indicate that the latter has sustained clinical effect.

Phentermine resin, a non-amphetamine appetite suppressant, does not appear to have an effect on reaction time at the recommended anoretic dose.

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SUMMARIO IN INTERLINGUA

Un Nove Methodo pro Mesurar le Effecto de Drogas Super le Performance de Subjectos Human

Es demonstrate que un machina que mesura quantitativemente le tempore de reaction in subjectos human pote esser usate pro studiar le effecto de drogas super ille function.

Alcohol, prendite in le forma de whiskey, prolonga le tempore de reaction, altera le curva de performance, e augmenta le numero del errores. Iste effectos pote esser mesurate quantitativemente.

Datos relative al effectos e al duration del effectos de amphetamina in le forma de sal solubile e in le forma de complexo a resina de excambio ionic indica que iste droga possede, in le secunde del duo mentionate formas, un sustenite effecto clinic.

Resina phenterminic, un suppressor de appetito non-amphetaminic, non pare haber un effecto super le tempore de reaction quando usate in le recommendate dose anoretic.

SUSCEPTIBILITY OF PROTEUS AND PROVIDENCE BACILLI TO 10 ANTIBACTERIAL AGENTS*

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With the introduction and ever increasing use of more and more antibacterial agents, Gram-negative bacilli now cause a larger proportion of bacterial infections than they did a decade ago (Finland, Jones and Barnes[8]). Prominent among these bacilli are *Proteus* species (Finland, Jones and Barnes[8], Yow[22,23]). Not only has the frequency of serious infections due to *Proteus* increased, but also *Proteus* and Providence species generally have a significant capacity to resist the action of many anti-bacterial agents. Noting that marked variation in susceptibility is common among *Proteus* strains, Yow[23] emphasized the need for careful *in vitro* testing of the susceptibility of *Proteus* species to various antibacterial agents by a tube dilution method. A systematic evaluation of the susceptibility of *Proteus* species to 10 antibacterial agents by a two-fold agar-plate dilution method was reported in 1954 by Potee, Wright and Finland[17]. Because the incidence of *Proteus* infections has continued to climb, and because new antibacterial agents have been introduced since 1954, the present study was undertaken to determine (a) whether any of the new antibacterial agents, on the basis of *in vitro* studies, is likely to be useful in the therapy of infections due to *Proteus* species, (b) whether any of the presently available antibacterial agents have significant *in vitro* effect on Providence group bacilli and (c) whether there is intra-species as well as inter-species variation in antibacterial agent susceptibility. While the clinical significance of (a) and (b) require no further comment, demonstration of consistency of susceptibility within a species would also have clinical application. For example, *Proteus mirabilis* does not produce indole from tryptophan. The simple laboratory test for indole permits recognition of the most common *Proteus* species; if there is no variation in susceptibility in this species, the therapeutic agent of choice can be predicted and therapy undertaken without delay for susceptibility testing.

The Providence group of bacilli have been considered to be intermediate between *Proteus* and *Salmonella-Shigella*, although biochemically and serologically they resemble *Proteus* more closely (Ewing, Tanner and Dennard[5], Stuart, Wheeler and Mc-

*This work was supported in part by grants 2E-39 and E-1792 from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service, Department of Health, Education and Welfare, Bethesda, Maryland.

Gann[21]). With the aid of a modified ferric chloride test (Falkow[6]), Providence group bacilli have been recognized with increasing frequency in the clinical microbiology laboratory of this hospital. All of the Providence isolates so far obtained from clinical specimens have been of pathogenic importance. For these reasons, susceptibility studies of 12 Providence isolates were carried out.

TABLE 1.—DIFFERENTIATION OF *PROTEUS* AND PROVIDENCE (Edwards and Ewing[3], Ewing, Tanner and Dennard[5], Falkow[6], Stuart, Wheeler and McGann[21])

	Reaction, T.S.I. agar						X	X		
ISOLATES	*Lactose*	*Glucose*	*Sucrose*	*H_2S*	*Urease Broth*	*Ferric chloride*	*Indole*	*Citrate*	*Mani-tol*	*Spread on BAP*
P. mirabilis	–	AG	– XX	+	+	+	–	+ XXX	–	+
P. vulgaris	–	AG	AG	+	+	+	+	±	–	+
P. morgani	–	AG	– XX	–	+	+	+	+	–	–
P. rettgeri	–	A °	– °°	–	+	+	+	+	A °°°	–
Providence	–	AG	A	–	–	+	+	+	–	±

X – Use 1% neopeptone and 0.5% l-trytophan broth.
XX – Ferments sucrose in 3 to 5 days, but negative in 24 hours on T.S.I. agar.
XXX – Usually negative, sometimes positive.
° – Acid production within 24 hours; small amount of gas in majority of instances.
°° – Ferments sucrose with prolonged incubation 5 to 30 days.
°°° – Usually negative.
A – Acid.
AG – Acid and gas.
BAP – Blood agar plate.
T.S.I. – Triple sugar iron.

Materials and Methods. Between February, 1959, and February, 1960, 95 isolates of *Proteus* and 12 isolates of Providence bacilli were obtained from clinical specimens submitted to the Clinical Microbiology Laboratory of the Salt Lake County General Hosital. Whenever possible, the clinical charts of the patients from whom the specimens were obtained were reviewed and retrospective decision as to pathogenic importance of each isolate was made.

IDENTIFICATION OF THE ORGANISM AND SPECIES. Isolation was carried out using MacConkey agar streak plates. Species of *Proteus* and Providence were identified on the basis of biochemical reactions as summarized in Table 1. Of the 95 isolates of *Proteus*, 52 were *P. mirabilis*, 20 were *P. rettgeri*, 15 were *P. morgani* and 8 were *P. vulgaris*. This distribution of *Proteus* species is that ordinarily seen in this hospital and is similar to that observed by others (Potee, Wright and Finland[17]).

For storage, isolates were incubated overnight in semi-solid nutrient agar in 13 × 100 mm. screw capped tubes and then stored at −22° until susceptibility studies were carried out.

ANTIBACTERIAL AGENTS*. Dilutions of chloramphenicol, cycloserine, erythromycin, strep-

*Chloramphenicol, dihydrostreptomycin, polymyxin B, crystalline potassium penicillin G and tetracycline were kindly supplied by Pfizer Laboratories; nitrofurantoin was supplied by Eaton Laboratories; erythromycin and cycloserine were furnished by Eli Lilly & Co.; kanamycin was supplied by Bristol Laboratories, Inc.; neomycin was obtained commercially.

tomycin, neomycin, polymixin B, tetracycline, kanamycin, nitrofurantoin, and penicillin were prepared, using commercial tryptic digest of casein-papaic digest of soybean broth. Sulfonamides were not included in this study, since clinical experience has indicated that sulfonamides are of little value in therapy of *Proteus* urinary tract infections regardless of the results of *in vitro* susceptibility tests (Kass[13]). The final concentrations in μg. (or units for penicillin) per ml., as used for testing, are listed in Table 2. At the time dilutions of an agent were prepared, 0.1 ml. aliquots of stock solutions in broth at 10 times the final test concentration were delivered to a series of 13 × 100 mm. screw capped culture tubes which were stored at −22° C., until used.

TABLE 2.–THE TEST CONCENTRATIONS EMPLOYED ARE INDICATED FOR EACH OF THE 10 ANTIBACTERIAL AGENTS TO WHICH CLINICAL ISOLATES OF *PROTEUS* AND PROVIDENCE BACILLI WERE EXPOSED

Antibacterial agents	*Final concentrations in μg. per ml. (Units per ml. for penicillin)*
Cycloserine Dihydrostreptomycin Neomycin Tetracycline Nitrofurantoin	0.1, 0.5; 1.0; 2.5; 5; 10; 25; 50; 100
Erythromycin Penicillin	0.1; 0.5; 1.0; 2.5; 5; 10; 25; 50; 100; 250; 500
Chloramphenicol	0.1; 0.5; 1.0; 2.5; 5; 10; 25; 50; 100; 200
Kanamycin	0.1; 0.5; 1.0; 2.5; 5; 10; 25; 50; 100; 250
Polymyxin B	0.05; 0.1; 0.5; 1.0; 2.5; 5; 10; 25; 50

TUBE DILUTION METHOD. For testing, one loopful of each isolate was transplanted from the semi-solid storage media to 10 ml. of freshly prepared tryptic digest of casein-papaic digest of soybean broth. After overnight incubation at 37° C., a direct bacterial count was done using the Petroff-Hausser chamber. On the basis of the count, the broth culture was diluted with an appropriate amount of tryptic digest of casein-papaic digest of soybean broth (with 0.5 mg. phenosulfonphthalein per 100 ml.) so that each 0.9 ml. contained 10,000 bacilli. The addition of such 0.9 ml. aliquots to tubes containing 0.1 ml. portions of the test dilutions of each antibacterial agent, resulted in a final volume of 1.0 ml. In this way uniformity of test inoculum and nutritional environment was assured. A control was included with each series by placing the 0.9 ml. of inoculum in a sterile tube containing 0.1 ml. of plain nutrient broth. After 20 to 24 hours of incubation at 37° C., the tests were read as follows:

1. Visible growth with turbidity and change in color of the phenol red was read as resistance.
2. Since absence of visible growth could result from either inhibition or death of the inoculated bacteria, subculture was required. After thorough mixing, a 3 mm. loopful of broth from each tube showing no growth was streaked on an eighth sector of a MacConkey agar plate which was then incubated for 24 hours at 37° C. The observed antibacterial action in broth was assumed to be bacteriostatic when growth occurred, and bactericidal when growth was absent on MacConkey agar subculture.

The validity of loop subculture for distinguishing bacteriostatic from bactericidal effect was tested by centrifuging 50 tubes in which there was no grossly visible growth for 60 minutes at 3000 rpm., 4° C. (r.c.f. at tube tip, 1300 g). The supernatant liquid was aspirated and discarded; 1 ml. sterile 0.9% sodium chloride solution was added. After mixing, the entire contents of each tube was decanted to a sterile petri dish; 20 ml. of sterile tryptic digest of casein-papaic digest of soybean agar (cooled to 56° C. after melting) were added and the contents mixed by swirling. After solidifying, the plates were incubated at 37° C. overnight. In each of the 16 instances where loop subculture gave growth, growth was also present in the corresponding pour plate; in each of the 34 instances where loop subculture gave no growth the corresponding pour plate was sterile.

Results. Of the 95 *Proteus* isolates studied, 79 were pathogenic as deter-

Gann[21]). With the aid of a modified ferric chloride test (Falkow[6]), Providence group bacilli have been recognized with increasing frequency in the clinical microbiology laboratory of this hospital. All of the Providence isolates so far obtained from clinical specimens have been of pathogenic importance. For these reasons, susceptibility studies of 12 Providence isolates were carried out.

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			XX			X	X			
P. mirabilis	–	AG	–	+	+	+	–	+	–	+
								XXX		
P. vulgaris	–	AG	AG	+	+	+	+	±	–	+
P. morgani	–	AG	–	–						
			XX		+	+	+	+	–	–
P. rettgeri	–	A •	– ••	–	+	+	+	+	A •••	–
Providence	–	AG	A	–	–	+	+	+	–	±

X – Use 1% neopeptone and 0.5% l-trytophan broth.
XX – Ferments sucrose in 3 to 5 days, but negative in 24 hours on T.S.I. agar.
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Erythromycin Penicillin	0.1; 0 5; 1.0; 2.5; 5; 10; 25; 50; 100; 250; 500
Chloramphenicol	0.1; 0.5; 1.0; 2.5; 5; 10; 25; 50; 100; 200
Kanamycin	0.1; 0.5; 1.0; 2.5; 5; 10; 25; 50; 100; 250
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1. Visible growth with turbidity and change in color of the phenol red was read as resistance.
2. Since absence of visible growth could result from either inhibition or death of the inoculated bacteria, subculture was required. After thorough mixing, a 3 mm. loopful of broth from each tube showing no growth was streaked on an eighth sector of a MacConkey agar plate which was then incubated for 24 hours at 37° C. The observed antibacterial action in broth was assumed to be bacteriostatic when growth occurred, and bactericidal when growth was absent on MacConkey agar subculture.

The validity of loop subculture for distinguishing bacteriostatic from bactericidal effect was tested by centrifuging 50 tubes in which there was no grossly visible growth for 60 minutes at 3000 rpm., 4° C. (r.c.f. at tube tip, 1300 g). The supernatant liquid was aspirated and discarded; 1 ml. sterile 0.9% sodium chloride solution was added. After mixing, the entire contents of each tube was decanted to a sterile petri dish; 20 ml. of sterile tryptic digest of casein-papaic digest of soybean agar (cooled to 56° C. after melting) were added and the contents mixed by swirling. After solidifying, the plates were incubated at 37° C. overnight. In each of the 16 instances where loop subculture gave growth, growth was also present in the corresponding pour plate; in each of the 34 instances where loop subculture gave no growth the corresponding pour plate was sterile.

Results. Of the 95 *Proteus* isolates studied, 79 were pathogenic as deter-

mined by review of the clinical charts of the patients from whom the isolates were cultured. Source and species correlation is provided in Table 3.

Seventy-one of the 79 pathogenic isolates were obtained by culture of the urine. Five of these specimens came from patients who had no evidence of renal involvement and were diagnosed as having cystitis. The remaining 66 patients with urinary tract infections due to *Proteus* were considered to have pyelonephritis. There was necropsy verification of this diagnosis in 3 cases. The clinical evidence for pyelonephritis in the remaining 63 patients consisted of: a) Urinary findings of pyuria and true bacteriuria (more than 100,000 bacteria per ml. urine) in each patient. b) Chills (20 patients), fever (42 patients), flank pain (7 patients). c) Urinary bladder catheterization was noted in the clinical records of 16 of these patients. d) Twenty-five had azotemia, 6 with uremia.

P. mirabilis was the most frequently encountered of the *Proteus* species causing urinary tract infections. Thirty-four (65%) of the 52 isolates of *P. mirabilis* were responsible for 52% (34 out of 66) of the cases of pyelonephritis. The high incidence of pyelonephritis due to *P. mirabilis* is probably a reflection of the high frequency of occurrence of *P. mirabilis* in urine cultures rather than of a particular renal pathogenicity. The other species, *P. rettgeri*, *P. morgani* and *P. vulgaris* were also quite capable of causing pyelonephritis; however, the over-all frequency of occurrence of these species was less than that of *P. mirabilis*.

Four pathogenic isolates of *Proteus* were obtained from blood cultures (2 *P. rettgeri*; 1 *P. morgani*; 1 *P. vulgaris*). One pathogenic isolate of *P. morgani* was isolated from an incisional abscess following nephrectomy.

Over half of the patients had therapy with one or more antibacterial agents. Sulfamethoxypyridazine, sulfisoxazole, nitrofurantoin, and chloramphenicol were most often used. Procaine penicillin, 600,000 units by intramuscular injection, was given twice daily to 6 patients with *P. mirabilis* infections; of the 6 isolates obtained from these patients during or after penicillin therapy, only one was resistant to penicillin by tube dilution susceptibility studies. However, according to penicillin disc susceptibility tests, using 10 unit discs, the highest concentration available in commercially offered discs, all of these 6 isolates were resistant to penicillin. Drugs of choice in the order of effec-

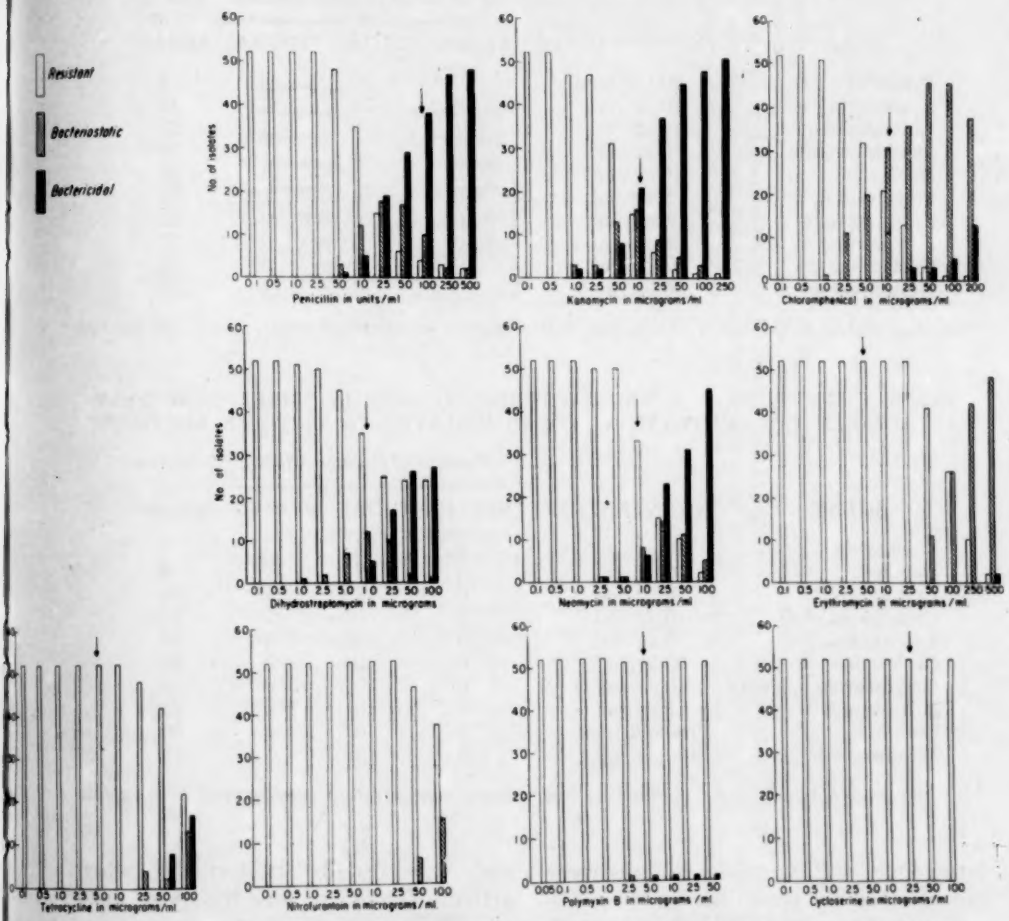
TABLE 3.—CORRELATION OF SPECIES WITH CLINICAL SOURCES AND PATHOGENICITY OF ISOLATES

		Sources of Isolates						*Diagnosis, patients with pathogenic isolates*			
Isolates	*No. of Isolates*	*Urine*	*Blood*	*Pus*	*Stool*	*Other*	*No. of Pathogenic Isolates*	*Pyelonephritis*	*Cystitis*	*Bacteremia*	*Abscess*
P. mirabilis	52	45	—	3	—	4*	42	34	5	—	3
P. rettgeri	20	18	2	—	—	—	19	17	—	2	—
P. morgani	15	11	1	1	2	—	12	10	—	1	1
P. vulgaris	8	7	1	—	—	—	6	5	—	1	—
Total	95	81	4	4	2	4	79	66	5	4	4
Providence	12	11	1		—	—	12	11	—	1	—

*3 from decubitus ulcers, and 1 from nasopharyngeal swab.

tiveness against *P. mirabilis* as indicated by the disc studies were neomycin, nitrofurantoin, chloramphenicol and streptomycin. On the other hand, the 43 non-*mirabilis* (indole positive) *Proteus* isolates were resistant to almost all antibacterial agents when evaluated by disc testing, except for neomycin; susceptibility was displayed by 30 of the 43 tested. Kanamycin discs were not used.

P. mirabilis. The results of tube dilution susceptibility testing of 52 isolates of *P. mirabilis* to 10 antibacterial agents are summarized in Fig. 1. The effectiveness of each antibacterial agent was judged on the basis of the average blood concentration of this agent which is obtainable in treating patients. In this way, rational comparison of the efficacy of the 10 agents tested is possible. Data relating effectiveness of



g. 1.—The height of the bars indicates the number of 52 clinical isolates of *Proteus mirabilis* which were sistant, inhibited or killed by various concentrations in broth of penicillin, kanamycin, chloramphenicol, ydrostreptomycin, neomycin, erythromycin, tetracycline, nitrofurantoin, polymyxin B and cylcoserine. e concentration indicated by the arrow was taken to be a therapeutically attainable blood concentran. Since the blood concentration of nitrofurantoin with conventional dosage is usually immeasurably low, no arrow could be placed on this part of the figure.

agents and *Proteus mirabilis* isolates are presented in Table 4. With *P. mirabilis*, penicillin was the most effective agent, since 100 units per ml., a therapeutically obtainable concentration, exerted a killing effect on 38 isolates and inhibited 10 additional isolates. Only 4 *P. mirabilis* isolates were not affected by penicillin.

Kanamycin, bactericidal versus 21 isolates and bacteriostatic versus 16 additional isolates, was effective against about three-fourths of the isolates of *P. mirabilis* tested. Chloramphenicol was bacteriostatic, the only effect demonstrated by this agent, with 31 isolates.

P. rettgeri. Test results with 20 isolates of *P. rettgeri* are summarized in Fig. 2 and Table 5. Kanamycin was the most effective agent of those tested and was usually bactericidal when active. Neomycin was the next most effective.

P. morgani. According to the data relating to 15 isolates of *P. morgani*, see Fig. 3 and Table 6, kanamycin was again the most effective agent. Neomy-

TABLE 4.—EFFECT OF 10 ANTIBACTERIAL AGENTS IN THERAPEUTICALLY ATTAINABLE CONCENTRATIONS ON 52 ISOLATES OF *PROTEUS MIRABILIS*

AGENT	*CONCENTRATION*	*Number of Isolates Affected*			*Number not Affected*
		STATIC	*CIDAL*	*TOTAL*	
Penicillin	100 units/ml.	10	38	48	4
Kanamycin	10 μg./ml.	16	21	37	15
Chloramphenicol	10 μg./ml.	31	0	31	21
Dihydrostreptomycin	10 μg./ml.	12	5	17	35
Neomycin	10 μg./ml.	8	6	14	38
Polymyxin B.	5 μg./ml.	0	1	1	51
Erythromycin	5 μg./ml.	0	0	0	52
Tetracycline	5 μg./ml.	0	0	0	52
Cycloserine	25 μg./ml.	0	0	0	52
Nitrofurantoin	100 μg./ml.*	15	0	15	37

*No measurable blood levels obtainable, but urinary concentration may exceed 100 μg./ml.

TABLE 5.—EFFECT OF 10 ANTIBACTERIAL AGENTS IN THERAPEUTICALLY ATTAINABLE CONCENTRATIONS ON 20 ISOLATES OF *PROTEUS RETTGERI*

AGENT	*CONCENTRATION*	*Number of Isolates Affected*			*Number not Affected*
		STATIC	*CIDAL*	*TOTAL*	
Kanamycin	10 μg./ml.	2	13	15	5
Neomycin	10 μg./ml.	4	7	11	9
Erythromycin	5 μg./ml.	0	1	1	19
Chloramphenicol	10 μg./ml.	1	0	1	19
Tetracycline	5 μg./ml.	0	0	0	20
Cycloserine	25 μg./ml.	0	0	0	20
Dihydrostreptomycin	10 μg./ml.	0	0	0	20
Polymyxin B	5 μg./ml.	0	0	0	20
Penicillin	100 units/ml.	0	0	0	20
Nitrofurantoin	100 μg./ml.	5	3	8	12

*No measurable blood levels obtainable, but urinary concentration may exceed 100 μg./ml.

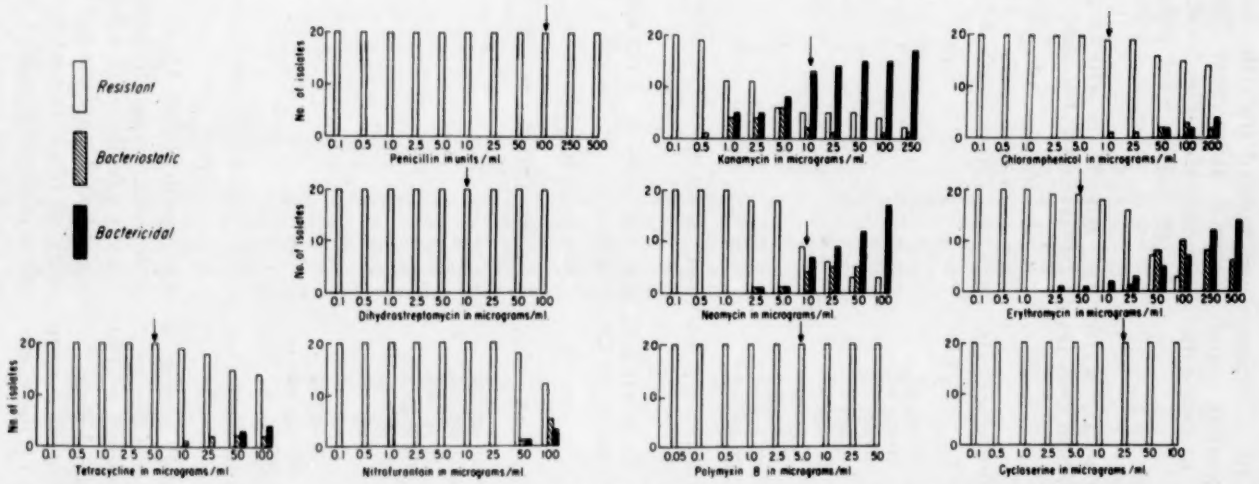


Fig. 2.—The height of the bars indicates the number of 20 clinical isolates of *Proteus rettgeri* which were resistant, inhibited or killed by various concentrations in broth of penicillin, kanamycin, chloramphenicol, dihydrostreptomycin, neomycin, erythromycin, tetracycline, nitrofurantoin, polymyxin B and cycloserine. The concentration indicated by the arrow was taken to be a therapeutically attainable blood concentration. Since the blood concentration of nitrofurantoin with conventional dosage is usually immeasurably low, no arrow could be placed on this part of the figure.

cin, effective against 9 isolates of *P. morgani,* was bactericidal about as often as it was bacteriostatic. Chloramphenicol, dihydrostreptomycin and tetracycline were usually bacteriostatic when effective.

Of these, 11 were cultured from patients with urinary tract infections; one was isolated from the heart blood of a patient at necropsy. Test results are summarized in Fig. 5 and Table 8. Of the 12 isolates of Providence bacilli,

TABLE 6.—EFFECT OF 10 ANTIBACTERIAL AGENTS IN THERAPEUTICALLY ATTAINABLE CONCENTRATIONS ON 15 ISOLATES OF *PROTEUS MORGANI*

AGENT	CONCENTRATION	Number of Isolates Affected			Number not Affected
		STATIC	CIDAL	TOTAL	
Kanamycin	10 μg./ml.	1	11	12	3
Neomycin	10 μg./ml.	5	4	9	6
Chloramphenicol	10 μg./ml.	8	1	9	6
Dihydrostreptomycin	10 μg./ml.	6	1	7	8
Tetracycline	5 μg./ml.	7	0	7	8
Penicillin	100 μg./ml.	2	1	3	12
Erythromycin	5 μg./ml.	0	1	1	14
Polymyxin B	5 μg./ml.	0	0	0	15
Cycloserine	25 μg./ml.	0	0	0	15
Nitrofurantoin*	100 μg./ml.*	6	2	8	7

*No measurable blood levels obtainable, but urinary concentration may exceed 100 μg./ml.

TABLE 7.—EFFECT OF 10 ANTIBACTERIAL AGENTS IN THERAPEUTICALLY ATTAINABLE CONCENTRATIONS ON 8 ISOLATES OF *PROTEUS VULGARIS*

AGENT	CONCENTRATION	Number of Isolates Affected			Number not Affected
		STATIC	CIDAL	TOTAL	
Kanamycin	10 μg./ml.	3	5	8	0
Neomycin	10 μg./ml.	4	0	4	4
Chloramphenicol	10 μg./ml.	4	0	4	4
Penicillin	100 units/ml.	1	2	3	5
Dihydrostreptomycin	10 μg./ml.	2	0	2	6
Erythromycin	5 μg./ml.	0	0	0	8
Tetracycline	5 μg./ml.	0	0	0	8
Polymyxin B	5 μg./ml.	0	0	0	8
Cycloserine	25 μg./ml.	0	0	0	8
Nitrofurantoin	100 μg./ml.*	2	0	2	6

*No measurable blood levels obtainable, but urinary concentration may exceed 100 μg./ml.

P. vulgaris. As presented in Fig. 4 and Table 7, kanamycin was the most effective of the agents tested against 8 isolates of *P. vulgaris.* Neomycin, chloramphenicol and penicillin were also active against some of these isolates.

Twelve isolates of Providence bacilli were obtained from clinical specimens. kanamycin killed 11 and inhibited 1 at a concentration therapeutically feasible. Penicillin was effective against about half the isolates and was bacteriostatic as often as bactericidal. Neomycin was effective against only one-third of the isolates.

Discussion. Study of the susceptibility of bacteria to anti-infectious agents

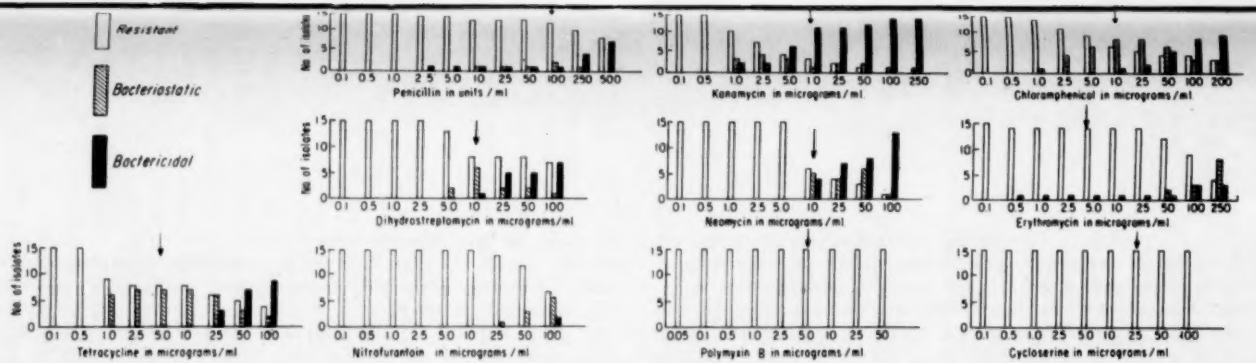

Resistant
Bacteriostatic
Bactericidal
No. of isolates
Penicillin in units / ml
Kanamycin in micrograms / ml
Chloramphenicol in micrograms / ml
Dihydrostreptomycin in micrograms / ml
Neomycin in micrograms / ml
Erythromycin in micrograms / ml
Tetracycline in micrograms / ml
Nitrofurantoin in micrograms / ml
Polymyxin B in micrograms / ml
Cycloserine in micrograms / ml

Fig. 3.—The height of the bars indicates the number of 15 clinical isolates of *Proteus morgani* which were resistant, inhibited or killed by various concentrations in broth of penicillin, kanamycin, chloramphenicol, dihydrostreptomycin, neomycin, erythromycin, tetracycline, nitrofurantoin, polymyxin B and cycloserine. The concentration indicated by the arrow was taken to be a therapeutically attainable blood concentration. Since the blood concentration of nitrofurantoin with conventional dosage is usually immeasurably low, no arrow could be placed on this part of the figure.

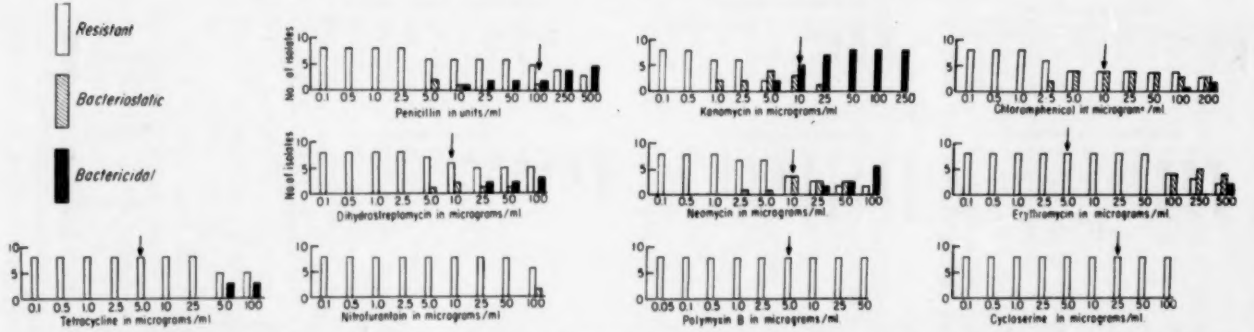


Fig. 4.—The height of the bars indicates the number of 8 clinical isolates of *Proteus vulgaris* which were resistant, inhibited or killed by various concentrations in broth of penicillin, kanamycin, chloramphenicol, dihydrostreptomycin, neomycin, erythromycin, tetracycline, nitrofurantoin, polymyxin B and cycloserine. The concentration indicated by the arrow was taken to be a therapeutically attainable blood concentration. Since the blood concentration of nitrofurantoin with conventional dosage is usually immeasurably low, no arrow could be placed on this part of the figure.

Fig. 5.—The height of the bars indicates the number of 12 clinical isolates of Providence bacilli which were resistant, inhibited or killed by various concentrations in broth of penicillin, kanamycin, chloramphenicol, dihydrostreptomycin, neomycin, erythromycin, tetracycline, nitrofurantoin, polymyxin B and cycloserine. The concentration indicated by the arrow was taken to be a therapeutically attainable blood concentration. Since the blood concentration of nitrofurantoin with conventional dosage is usually immeasurably low, no arrow could be placed on this part of the figure.

is best done in liquid media. Every bacterium of a given test inoculum is exposed to the same concentration of the test antibacterial agent. Moreover, when different agents are compared, variations in diffusibility, a characteristic dependent on many factors including molecular size and charge, are eliminated. Furthermore, bactericidal can be distinguished from bacteriostatic action. These attributes have been commented on by several authors (Eagle, Levy and Fleischman[2], Schneierson and Amsterdam[19]).

variations in apparent susceptibility. Bacteria would appear to be particularly resistant when a large inoculum is exposed to a small concentration of anti-infectious agent. Should the bacterium under study be relatively resistant to the action of the agent, inoculum size would assume even more critical significance.

The efficacy of penicillin against *Proteus* microorganisms was apparently first reported by Helmholz and Sung[11] in 1944. A systematic study, with species identification of *Proteus*, was

TABLE 8.—EFFECT OF 10 ANTIBACTERIAL AGENTS IN THERAPEUTICALLY ATTAINABLE CONCENTRATIONS ON 12 ISOLATES OF PROVIDENCE BACILLI

AGENT	CONCENTRATION	Number of Isolates Affected			Number not Affected
		STATIC	CIDAL	TOTAL	
Kanamycin	10 μg./ml.	1	11	12	0
Penicillin	100 units/ml.	4	2	6	6
Neomycin	10 μg./ml.	2	2	4	8
Chloramphenicol	10 μg./ml.	0	0	0	12
Dihydrostreptomycin	10 μg./ml.	0	0	0	12
Erythromycin	5 μg./ml.	0	0	0	12
Tetracycline	5 μg./ml.	0	0	0	12
Polymyxin B	5 μg./ml.	0	0	0	12
Cycloserine	25 μg./ml.	0	0	0	12
Nitrofurantoin	100 μg./ml.*	3	0	3	9

*No measurable blood levels obtainable, but urinary concentration may exceed 100 μg./ml.

Uniformity of concentration of antibacterial agent is assured by tube dilution testing. Inoculum size, the other side of the coin, must also be carefully controlled to standardize testing. When bacteria, whether susceptible or resistant, are exposed to either penicillin or streptomycin, a finite mass of the test agent is irreversibly fixed by the bacterial cells (Eagle, Levy and Fleischman[2], Linton[14]). By inference, binding occurs with other anti-infectious agents as well, particularly when these agents exert a demonstrable antibacterial effect. Consequently, variations in inoculum size involving several orders of magnitude might well be reflected in

reported by Potee, Wright and Finland[17] in 1954, and disclosed that, in general, the penicillin susceptible *Proteus* were *P. mirabilis*, while the other 3 (indole-producing) species were penicillin resistant. The present report affirms the effectiveness of penicillin against *P. mirabilis*.

In addition, 3 of the 8 isolates of *P. vulgaris* studied were susceptible to penicillin in therapeutically obtainable concentrations. Garrod, Shooter and Curwen[10], reported both *P. morgani* and *P. vulgaris* were frequently susceptible to penicillin at concentrations of 5 units or less per ml., after study of 81 and 63 isolates respectively.

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Dihydrostreptomycin	10 μg./ml.	0	0	0	12
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Of the 52 isolates of *P. mirabilis* included in the present study, 35 were tested with 10 unit penicillin discs. All 35 were resistant by this test, indicating the necessity for tube dilution testing for demonstration of clinically significant susceptibility to penicillin.

Hewitt and Finegold[12] reported kanamycin was effective against *P. rettgeri* and *P. morgani* as well as *P. mirabilis*. In marked contrast, Eisenberg and coworkers[4] found that no species of *Proteus* was susceptible to kanamycin in concentrations less than 20 micrograms per ml. The data of the present study indicate kanamycin is the most effective of the 10 antibacterial agents tested against all 3 of the indole-positive species of *Proteus* and is second only to penicillin in effectiveness against *P. mirabilis*. In the study of Eisenberg, Weiss and Flippin[4], various concentrations of kanamycin in agar petri-plate cultures were inoculated with unspecified, but large numbers of test *Proteus*. It may well be that the poor showing of kanamycin versus *Proteus* species in this setting relates to the poor diffusibility of kanamycin through agar and the large inoculum of test bacilli applied to the agar. Clinical experience with kanamycin therapy of *Proteus* infections, although limited in extent, has been favorable in Bunn's experience (Bunn, Baltch and Krajnyak[1]). Neomycin and kanamycin display nearly complete cross resistance and susceptibility. On a mass basis, kanamycin has been found to be less active than neomycin against staphylococci, but more active against *Proteus* species (Finegold[7]). In the present study, neomycin was effective against only a few *P. mirabilis* isolates, however, neomycin was second only to kanamycin in effectiveness against *P. rettgeri, P. morgani,* and *P. vulgaris*.

That *Proteus* species varied in susceptibility to chloramphenicol was observed by Schoenbach[20] in 1949. Some increase in resistance of *Proteus* species to chloramphenicol was noted by Potee, Wright and Finland[17], after comparing the susceptibility of *Proteus* isolated in 1954 with *Proteus* obtained in 1949 (Frank, Wilcox and Finland[9]). Of the 1954 isolates, *P. mirabilis* strains were generally resistant to 12.5 μg. per ml. Chloramphenicol (10 μg. per ml.) inhibited about one-half of the isolates of *P. mirabilis, P. morgani* and *P. vulgaris* of this study.

Tetracycline, at a clinically obtainable concentration, inhibited only 7 *P. morgani* isolates among the entire 95 isolates of *Proteus* tested in this study. Insusceptibility of *Proteus* to tetracycline in 1959 is in keeping with the results of Potee, Wright and Finland[17], who described a definite, significant increase in resistance of Proteus to tetracycline, comparing isolates of 1949 with those of 1954.

Dihydrostreptomycin was effective against about one-half of the 15 *P. morgani* and about one-third of the 52 *P. mirabilis* isolates; occasionally, it was effective against *P. vulgaris* but was completely ineffective against *P. rettgeri*. By earlier *in vitro* studies (Petersdorf, Bennett and Rose[16]), streptomycin was quite ineffective against all *Proteus* species, if the results were interpreted in terms of therapeutically obtainable blood levels.

In the present study, nitrofurantoin was only occasionally effective at a concentration of 100 μg. per ml. or higher, a finding in conflict with the results reported by Middleton[15]. The clinical study, reported by Richards *et al.*[18], indicated that nitrofurantoin failed to achieve permanent clearing of urinary tract infections due to *Proteus* species. It is probably that in part this lack of good effect relates to lack of adequate blood or "tissue" concen-

trations of nitrofurantoin with usual clinical dosage (Kass[13]).

The marked resistance of *Proteus* to erythromycin and polymyxin B observed in this study was also noted by Potee, Wright and Finland[17] in 1954.

Cycloserine, *in vitro,* was completely ineffective against *Proteus* species. No data were found relating to clinical experience with cycloserine in therapy of *Proteus* infections.

Of the 10 agents tested, kanamycin was the most effective against Providence isolates. While 12 isolates do not constitute a large experience, no comparable data regarding the susceptibility of clinical specimen isolates of Providence bacilli were found.

Summary. By means of a carefully standardized tube dilution method, 95 clinical isolates of *Proteus* (representative of the usual United States species distribution) and 12 clinical isolates of Providence group bacilli were tested for susceptibility to 10 anti-infectious agents. Each agent was tested at a number of concentrations. However, the effectiveness of each agent was judged at the test concentration corresponding to the blood concentration attainable in patients.

Penicillin was the most effective agent against *P. mirabilis;* kanamycin was the most effective agent against *P. rettgeri, P. morgani, P. vulgaris* and Providence bacilli.

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(Interlingua on page 302)

CURRENT OBSERVATIONS IN PNEUMOCOCCAL PNEUMONIA: I. AZOTEMIA AS A PROGNOSTIC OMEN

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The prognosis in pneumococcal pneumonia is alluded to in the prognostic aphorisms on pleurisy by Hipprocates[4] and later well observed from the clinical standpoint by Osler[16] in 1892. Prognostic observations of this disease are further listed in most texts or treatises on the subject (Austrian and Austrian[1], Finland[7], LaDue[12], Myers[15]). Although well-known to the experienced clinician, as far as we are aware, little has been written on the prognostic value and application of coexisting azotemia in this disease.

The purpose of this paper is to report the observation that in patients with pneumococcal pneumonia, still a fatal disease and probably under-estimated in the current penicillin era, a normal blood urea nitrogen or non-protein nitrogen content of the blood favors a good prognosis: whereas, an azotemia which is severe and does not decline may be ominous from the prognostic standpoint. The necropsy findings from the kidney will be discussed briefly.

Materials and Methods. A series of deaths from pneumococcal pneumonia with azotemia prompted this study originally. All charts indicating the presence of pneumococcal pneumonia in patients at the Veterans Administration Medical Teaching Group (Kennedy) Hospital between 1957 and 1959 were reviewed. Those with a blood urea nitrogen (BUN) were abstracted. Also the charts on all patients with positive blood cultures for the *Diplococcus pneumoniae* between December 29, 1954, and April 2, 1960, with or without a BUN, were abstracted as well.

The necropsy findings of 4 additional cases from the John Gaston Hospital, University of Tennessee College of Medicine, are also presented separately with the pathologic findings.

The criteria for the diagnosis of pneumococcal pneumonia was probably over-strict as bacteriologic culture proof was required in

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addition to the pulmonary infiltration on the roentgenogram of the chest. Each chart was then reviewed and those meeting these rather strict criteria for diagnosis of pneumococcal pneumonia were then abstracted, tabulating the clinical, roentgenographic, laboratory, and survival-mortality findings. This paper will be limited primarily to the presence or absence of azotemia and the correlation with the survival or mortality. A brief mention will be made also of the sero-types of the strains of *Diplococcus pneumoniae* grown from the blood culture with reference to mortality and the racial incidence.

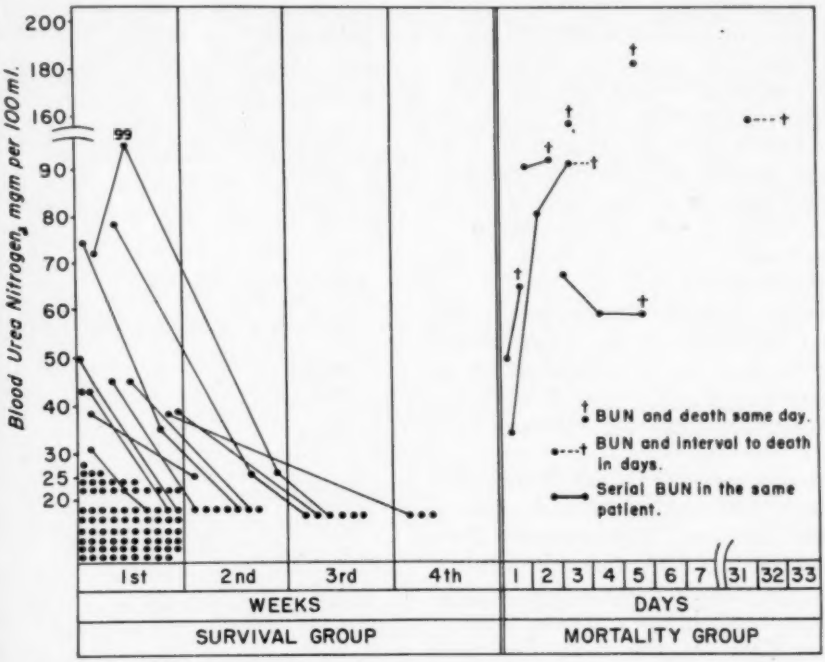

Fig. 1.—Chart tabulating relationship of the blood urea nitrogen in the survival group and mortality group of patients at the Veterans Administration Medical Teaching Group Hospital with pneumonia caused by the *Diplococcus pneumoniae*. See text for selection of patients.

Results. RELATIONSHIP OF THE BUN TO SURVIVAL-MORTALITY. Figure 1 tabulates the relationship of the BUN to the survival-mortality for the patients from the Veterans Administration Medical Teaching Group (Kennedy) Hospital (VAMTG Hospital). Of the 96 survivors, 70 had a BUN that was 25 mg. per 100 ml. or less first obtained, usually shortly after hospital admission. All BUNs in the survival group, if otherwise elevated initially had a tendency to fall serially to less than 25 mg. per 100 ml. with one exception, and this was in a patient with Type III pneumococcal pneumonia with a positive blood culture who had a BUN on admission of 71 mg. per 100 ml., rose 2 days later when he was critically ill to 99 mg. per 100 ml., and then dropped to 50 mg. per 100 ml. on the fifth day during which time he had improved, and then progressively dropped to 25 mg. per 100 ml. the second week and to 18 mg. per 100 ml. shortly thereafter.

All those who died had BUNs that were elevated or continued to remain so and usually rising. This group as

tabulated were cases of pneumococcal pneumonia without other known significant bacterial infections.

It should be mentioned for the sake of completeness that in 2 other patients with pneumococcal disease who died, the BUN was 19 mg. per 100 ml. in one and 24.9 mg. per 100 ml. in another, but both died on the second hospital day of a severe pneumococcal meningitis without significant pneumonia. A third death of pneumococcal disease with positive blood culture for *Diplococcus pneumoniae* with pneumonia died with a mild elevation of BUN to 37 mg. per 100 ml.; this case was complicated by a severe meningitis in which staphylococcus was grown almost as a pure culture from the brain at the time of necropsy.

The deaths from pneumococcal pneumonia infections at the John Gaston Hospital were similar to those that were observed at the Veterans Administration Medical Teaching Group (Kennedy) Hospital.

POSITIVE BLOOD CULTURES. A record of 66 cases with positive blood cultures for the *Diplococcus pneumoniae* from December 29, 1954, through April 2, 1960, are tabulated with the Neufeld sero-types (Table 1). There were 6 deaths. Two of the 6 deaths were among 3 patients with Type III bacteremia and one who survived had the stormiest course, clinically, and had the BUN changes described above. Among the other deaths there was one each of Type I, Type II, Type XIV and Type XXIII.

Among the patients with positive blood cultures, 23 were white and 43 Negroes, and there were 15 severe alcoholics in each racial group. Of the deaths 2 were in white and 4 in Negroes. These cases are from the VAMTG Hospital; the admissions to that hospital are also shown for the period in Table 2.

PATHOLOGY. Necropsy material was available in 11 of the cases in the present series. Seven of the cases were from the VAMTG Hospital and 4 from the John Gaston Hospital. In each of these cases the sections of the kidneys were reviewed, and an attempt was made to evaluate changes affecting the glomeruli, tubules, arteries, arterioles, and interstitial tissue both qualitatively and quantitatively.

TABLE 1.—VETERANS ADMINISTRATION MEDICAL TEACHING GROUP (KENNEDY) HOSPITAL. INCIDENCE OF POSITIVE BLOOD CULTURE TYPES FOR *DIPLOCOCCUS PNEUMONIAE* AND DEATHS

December 29, 1954, to April 2, 1960

Type	*No. of Cases*	*Deaths*
I	14	1
II	7	1
III	3	2
IV	1	
V	1	
VI	1	
VII	10	
VIII	5	
XII	5	
XIV	4	1
XVI	1	
XVII	2	
XVIII	1	
XIX	2	
X	1	
XIII	2	1
XV	1	
Unclassified	5	
Total	66	6
Crude Mortality		9%

The interval between the time of death and the performance of the necropsy varied from one to 16 hours. All tissues were fixed in 10% formalin. The sections were stained with both hematoxylin and eosin and PAS. In addition, Gram stains and iron stains were prepared in many instances.

Varying degrees of ischemic changes

of glomerular tufts, cloudy swelling and hyaline droplet degeneration of tubular epithelium, tubular casts, arterial and arteriolar nephrosclerosis, and round cell infiltration of the interstitial tissues were noted. Nephrosclerosis, cloudy swelling of the tubular epithelium, and the presence of tubular casts were noted in many of the cases. However, there was no one change, or group of changes common to all of the cases either qualitatively or quantitatively. The vascular disease encountered was consistent with the age of the patients and their previous clinical course.

The degree of cloudy swelling of the tubules did not correlate with the time interval between death and necropsy or the level of the BUN in these cases. The cloudy swelling was not considered to differ in degree from that associated with any case having a febrile and septic course terminating in death. Since soluble exotoxins, capable of producing tissue damage, have not been described with the pneumococcus (Dubos[5]), the tubular change is not considered a specific one, and is possibly due to accelerated autolysis due to the febrile state at the time of death.

On the basis of the findings in our cases, we have concluded that there are no specific kidney lesions in cases having an elevated BUN and dying of pneumococcal pneumonia: the azotemia appears to be primarily on a prerenal basis. However, it may well be enhanced by a loss of functioning reserve of the kidney due to pre-existing vascular or inflammatory disease. A complete analysis of the case material in this paper indicated the deaths were in the "poor risk" category of patients where usually 3 or more of the following prognostic contributing factors in each death case were present: age, alcoholism, dehydration, nutritional status, type of prior and subsequent treatment, or complications or other underlying disease, serotype of the organism, leukocyte response, and the extent of the disease.

TABLE 2.—VETERANS ADMINISTRATION MEDICAL TEACHING GROUP (KENNEDY) HOSPITAL. INCIDENCE OF RACE AND DEATH RATES IN PATIENTS WITH POSITIVE BLOOD CULTURE FOR *DIPLOCOCCUS PNEUMONIAE*

December 29, 1954, through April 2, 1960

	Patients with Positive Blood Cultures			*Hospital Admissions*	
	No.	*%*	*No. of Deaths*	*No.*	*%*
White	23	(35%)	2	36,305	(72%)
Negro	43	(65%)	4	14,300	(28%)

Discussion. Previous studies on renal physiology during acute pneumococcal pneumonia have yielded variable results (Farr and Abernethy[6]). An apparent increase of the functional capacity of the kidney during pneumonia was reported by Lewis[13], McIntosh and Reimann[14], and Goldring[10]. Neither impairment of function nor hyperfunction was reported by Rackemann, Longcope, and Peters[18], and Frothingham[9]. On the other hand Tileston and Comfort[22], Schwartz and McGill[20] and Bookman[3] reported definite evidence of renal impairment. The association of the *Diplococcus pneumoniae* with the nephrotic syndrome is reported by Blackman[2] and with nephritis by Rake[19] and Seegal[21]. In this regard Rake[19] reported that *Diplococcus pneu-*

moniae was second to the streptococcus as a bacterial incitant of Bright's disease. However, Austrian and Austrian[1] felt that observations of increase of blood urea nitrogen usually led to erroneous conclusions concerning the status of the kidney in this disease.

Thus, in view of the discordant results of early investigations, Farr and Abernethy[6] carefully studied the renal function in 28 cases of pneumococcal pneumonia. The urea clearance test indicated an actual increase in this type of renal function in the pre-critical stage of disease, particularly in the younger age group (up to the age of 40). They concluded that in the pre-critical phase of pneumonia, renal function may be elevated, unchanged, or slightly impaired. In general, they noted the level of renal activity was inversely proportionate to the age of the patient. Of particular interest are 2 cases in this series which exhibited high BUN on admission with low urea clearance, both of which returned to normal in the next few days.

In the discussion of the syndrome of extrarenal azotemia, Jeghers and Bakst[11] reported a case of pneumococcal pneumonia with a positive blood culture for Type V in which the NPN was 75 mg. per 100 ml., with death 2 days after admission with necropsy indicating hydropic changes in the renal tubules with granular debris in some of the tubular lamina and the glomeruli appearing grossly normal. It was their opinion that increased protein catabolism, dehydration, electrolyte disturbance, lowered blood pressure, and toxic damage to the adrenals and kidneys were significant factors producing azotemia in pneumonia. To this Finland[8] adds age of the patient, state of nutrition, liver failure, and cardiac status as contributory factors; also, tetracycline drugs may produce some rise in BUN in certain individuals.

Azotemia has prognostic significance in other severe infectious diseases, such as typhus fever (Yeomans[24]) and plague. Thus, this observation is not implied to apply alone to pneumococcal pneumonia.

Parrish, Rubenstein and Howe[17] observed several patients who had onset of azotemia and simultaneous respiratory infection. They had noted that renal pathology associated with acute renal insufficiency involving both the glomeruli and tubules, frequently referred to as the "lower nephron syndrome," which occasionally accompanies acute infections, appeared to be infrequently associated with respiratory infections. Three of these patients were studied utilizing the inulin-sodium para-aminohippurate clearances and needle biopsy of the kidneys. In 3 of the patients with pulmonary signs suggesting pneumonia, renal biopsy showed marked tubular changes which was confirmed on function studies and which appeared to be reversible. Glomerular damage was also demonstrated on the function studies, but this improved. The etiology of the renal changes was considered to be obscure and they believed that the combination of dehydration, pulmonary infection, and alcoholism in their 3 patients was significant. From our experience it is apparent that the azotemia in pneumococcal pneumonia is non-specific but appears to have prognostic significance.

Thus, to predict the outcome in any given case of pneumococcal pneumonia or to evaluate therapeutic results in any series, it is necessary to take into consideration many factors that influence the prognosis. Wood[23] points out the prognosis of pneumococcal pneumonia is influenced adversely by the presence of old age (also infancy), late treatment, infections with certain types of pneumococcus (particularly Types II and III), involvement of more

than one lobe of the lung, leukopenia, bacteremia, jaundice, presence of complications (notably shock and meningitis), pregnancy (particularly in the third trimester), presence of other disease such as heart disease, diseases of the lung, and so forth, and alcoholism. To this group, especially if persistent and increasing, azotemia appears to have its place in the list of factors influencing prognosis in pneumococcal pneumonia.

Summary. Data has been presented relating the prognostic application of simple blood chemical determination, the blood urea nitrogen, as an additional prognostic aid evaluating the patient with pneumococcal pneumonia. The results of this study indicate that azotemia in the presence of pneumococcal pneumonia is non-specific and probably reflects the response of the host to a variable number of factors, predominantly pre-renal, usually superimposed on a variable degree of pre-existing renal disease and other prognostic factors discussed.

In terms of presence or absence of azotemia, our study indicates that patients who survived pneumococcal pneumonia either had a normal BUN, or if the BUN was elevated at the time of admission to the hospital, it declined to 25 mg. per 100 ml. or less. Those patients who died of pneumococcal pneumonia, uncomplicated by other bacterial infections, had a blood urea nitrogen that was elevated and usually remained elevated. However, this prognostic observation does not apply to acute pneumococcal meningitis without significant pulmonary involvement or in the presence of other complicating infections.

ACKNOWLEDGMENT: Appreciation is expressed to Dr. Maxwell Finland of Harvard Medical School for his initial helpful suggestions and review of this paper. Appreciation also is expressed to the two cooperating hospitals for their permission to review their charts in obtaining the data for this paper.

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SUMMARIO IN INTERLINGUA

Observationes Currente in Re Pneumonia Pneumococcal: I. Azotemia Como Augurio Prognostic

Es presentate datos relative al application prognostic de un simple determination de chimia del sanguine, i.e. le determination del nitrogeno de urea del sanguine, como adjuta prognostic additional in le evalutation del patiente con pneumonia pneumococcal. Le resultatos de iste studio indica que azotemia in le presentia de pneumonia pneumococcal es non-specific e reflecte probabilemente le responsa del hospite a un variabile numero de factores, de character predominantemente pre-renal, que es usualmente imponite super un variabile grado de pre-existente morbo renal e de altere factores prognostic que es discutite in le presente communication.

Quanto al presentia o absentia de azotemia, nostre studio indica que patientes qui superviveva a pneumonia pneumococcal habeva (1) valores normal de nitrogeno de urea del sanguine o (2), si le nivello del nitrogeno de urea del sanguine esseva elevate al tempore del hospitalisation, un reduction de ille valores a 25 mg per 100 ml o minus. Le patientes qui moriva de pneumonia pneumococcal in le absentia de complicationes per altere infectiones bacterial habeva valores pro le nitrogeno de urea del sanguine que esseva elevate e que usualmente remaneva elevate. Tamen, iste observation prognostic non es applicabile a acute meningitis pneumococcal sin significative affection pulmonar. Similemente illo non es applicabile a casos characterisate per le presentia de altere infectiones complicatori.

ABNORMAL HEMOGLOBINS.
II. THE PATHOLOGY OF SICKLE CELL TRAIT*†

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In a continuing study underway in this department over the past 31 months, 135 cases of sickle cell disease in patients over one month of age have been examined at necropsy. This paper describes in detail the findings in the largest, but probably least studied group, namely, those subjects with sickle cell trait. It is not proposed to discuss sickle cell trait exhaustively from an historical or literature review aspect, but rather to let the facts obtained in this study speak more-or-less for themselves.

Methods and Materials. During the period of study, 3079 necropsies were performed at this institution. From this large group we collected samples from 2,011 cases, or 65%. Of the 35% of cases not collected, the majority (about 20%) were in still-born and newborn infants. Out of the 2,011 samples collected, 1248 were from Negroes over one month of age. It is this group that forms the material used in this study.

Ten milliliters of blood were removed from the hearts of patients coming to necropsy at the Institute of Pathology, University of Tennessee, Memphis. This blood was treated according to the method previously described (McCormick[13]), and aliquots used for hemoglobin paper electrophoresis, sodium metabisulfite sickle cell preparations, and alkali denaturation (Singer, Chernoff and Singer[21]). In addition, approximately 30 tissue sections were taken from each necropsy, fixed in 10% neutral formalin, embedded in paraffin, sectioned at a thickness of 5 microns, and stained with hematoxylin and eosin. Special stains for iron, fibrous tissue, and so forth, were used when necessary.

Exact quantitation of the amount of S hemoglobin present was done on some, but not all, of these cases. The Spinco Analytrol was used for this quantitation. Gross quantitation of all cases was done, and only those with a definite A-S pattern used.

In addition, 2660 blood specimens were obtained from unselected clinical patients at the City of Memphis Hospitals, West Tennessee Tuberculosis Hospital, and Western State Hospital. This large number of cases served as a control for the necropsy study.

Results. One hundred and twenty cases of sickle cell trait have been found at necropsy during the past 31 months (Table 1). These patients ranged in age from one month to 90 years, and averaged 47.3 years. This mean age was not significantly different from that of the 1,104 Negro patients with normal (A-A) hemoglobin (47.4 years). No significant sex difference was noted. All of these cases of sickle cell trait were in Negroes.

Every case in which sickle cell hemoglobin could be demonstrated on electrophoresis was found to contain sickled cells in the fixed tissues. The degree of sickling varied considerably from case to case, independent of age, sex, or the quantity of hemoglobin S present (McCormick[13]).

In 15 of the 120 cases (12.5%) truly massive intravascular sickling unassociated with any infarcts was seen. An additional 22 cases (18%) contained one or more obvious visceral infarcts

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thought to be due only to the sickle cell trait (Table 2). In comparison, less than 1.0% of patients with A-A hemoglobin had visceral infarcts not due to emboli from bacterial endocarditis or cardiac mural thrombi, or to atherosclerosis. In the remaining 83 cases sickled cells were obvious, but were not in massive numbers or associated with visceral infarction.

The criteria used in this study for the diagnosis of sickle cell trait are given in Table 3.

The seasonal incidence of crises in sickle cell anemia (S-S) usually associated with acute respiratory tract infections is well known (Wright and Gardner[26]), but no mention of such an occurrence has been published concerning the trait. When the month in which death occurred is plotted for these 120 cases, no obvious seasonal difference is noted (Fig. 1). The peak in March, which occurred in all 3 years this study has been underway, may represent a true increased death incidence or could be coincidence.

In the 120 cases of sickle cell trait

TABLE 1.—INCIDENCE OF SICKLE CELL GENE IN MEMPHIS AND WESTERN TENNESSEE*

Source	*Race*	*No. Examined*	*Sickle Cell Trait (A-S)*	*Totals (A-S, S-S, S-C)*
Necropsies	Negro	1248	120 (9.6%)	135 (10.8%)
	White	358	0 (0%)	0 (0%)
Clinical	Negro	1660	140 (8.4%)	150 (9.0%)
	White	1000	0 (0%)	0 (0%)
Totals	Negro	2907	260 (9.0%)	285 (9.8%)
	White	1358	0 (0%)	0 (0%)

*Patients, both necropsy and clinical, are from the City of Memphis Hospitals, West Tennessee Tuberculosis Hospital, and Western State Hospital. Necropsies on 405 additional infants less than one month of age not included in this series. Six cases of proven sickle cell disease were seen in this group.

TABLE 2.—SITE OF VISCERAL INFARCTS (120 CASES OF A-S)*

Spleen	
Old	3
Fresh	8
(Perifollicular hemorrhages—18 additional cases)	
Lungs	1
Brain	2
Pituitary	2
Adrenals	2
Kidneys	2
Large and small bowel	2
Liver	1
Prostate	1
	22 cases (18%)

*No cases in which cardiac mural thrombi, bacterial endocarditis, or severe atherosclerosis are included. In all of these cases infarction is thought to be due only to sickle cell trait. Two of these cases contained multiple infarcts but are counted as only one case each.

comprising this study, death was thought to be due primarily to the trait in 5 cases (4.2%), and to be a major contributing factor in 10 others (8.3%) (Table 4). Thus, in 12.5% of cases, the trait caused death or greatly contributed to it. If one were to include all cases of massive generalized sickling and visceral infarction to this group, 25 additional cases, or a total of 40 cases (33%) would be found.

Discussion. The findings in this extensive necropsy series of sickle cell trait patients demonstrate the fre-

TABLE 3.—CRITERIA FOR DIAGNOSIS OF SICKLE CELL TRAIT

1. Positive sodium metabisulfite sickle cell preparation.
2. A-S pattern on paper electrophoresis, with the major component being hemoglobin A.
3. Less than 2% fetal hemoglobin on alkali denaturation in all cases over 6 months of age.
4. Presence of sickled erythrocytes in formalin fixed tissues.
5. Absence of splenomegaly at necropsy*, and absence of siderofibrotic nodules.
6. Clinical data (absence of microspherocytes or large numbers of target cells).

*Average spleen weight at necropsy of adult Negroes with A-A hemoglobin 101 ± 12 gm. All cases of leukemia-lymphoma, severe congestive heart failure, cirrhosis with congestive splenomegaly, and severe splenic artery atherosclerosis omitted. The A-S spleens did not differ significantly from this range.

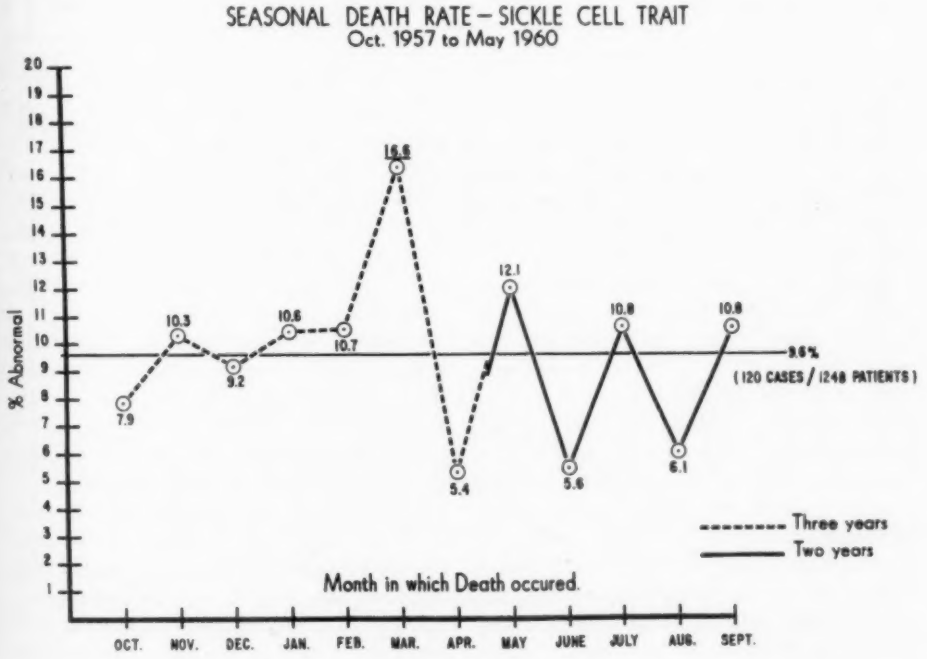

Fig. 1

TABLE 4—CASES IN WHICH A-S CAUSED OR WAS A MAJOR FACTOR IN DEATH

	Age, Race and Sex	*Disease(s) Present at Necropsy*	*Finding Relative to Sickle Cell Trait*
Sickle Cell Trait Responsible For Death	1. 2½ mo. CM	Pulmonary edema	Massive generalized sickling
	2. 8 mo. CM	Mild interstitial pneumonitis	Massive generalized sickling
	3. 4 mo. CM	Mild interstitial pneumonitis	Massive generalized sickling
	4. 2 CF	Mild diarrhea	Massive generalized sickling
	5. 32 CF	Pulmonary edema, mild	Massive generalized sickling
Sickle Cell Trait Probably a Major Contributing Factor in Death	1. 2 mo. CF	Pertussis	Massive sickling
	2. 82 CF	50% 2° body burns	Massive sickling
	3. 70 CM	Post-spinal anesthesia (for T.U.R.)	Massive generalized sickling and multiple infarcts
	4. 40 CM	Staph. food poisoning with shock	Massive sickling
	5. 40 CF	Anemia and congestive heart failure	Massive sickling
	6. 51 CM	Bronchial asthma	Massive sickling with perifollicular hemorrhages in spleen
	7. 51 CM	Alcoholism (0.26%)	Generalized massive sickling
	8. 54 CF	Acute and chronic tracheobronchitis, moderate	Massive sickling and visceral infarcts
	9. 8 mo. CF	Pillow found over head	Massive sickling
	10. 2 mo. CF	Otitis media and minimal bronchopneumonia	Massive sickling

quency of unequivocal antemortem visceral infarcts and massive generalized sickling associated with minimal or no other findings. The mean age of these 120 patients was not significantly different from the normal hemoglobin control cases. In addition, no definite seasonal death rate was observed. This differs from the observation of seasonal peaks in crises in patients with sickle cell anemia (Wright and Gardner[26]).

The universal presence of sickled cells in the tissues of these 120 A-S patients demonstrates that this disease can always be detected at necropsy. The rather wide range in the degree of sickling, and the varying morphology of the individual sickled cell makes truly accurate differentiation between the various S hemoglobinopathies impossible, in spite of the opinion of others concerning the accuracy of diagnosis using degree of sickling and the shape of the sickled cells (Mostofi, Vorder Bruegge and Diggs[16]). A more complete discussion of this problem has been given in an earlier paper (McCormick[13]).

The question of selectivity of material in such a study as this always arises. Some aspects of this problem have been discussed elsewhere[13]. The statistical difference in the incidence of sickle cell trait in the necropsy study and clinical control study is not significant ($p = 0.20$ to 0.30).

Examination of Table 4 reveals how otherwise trivial diseases, such as diarrhea with moderate dehydration (Case 4) or alcoholic intoxication (Case 7) can cause massive sickling and lead to death. Alcoholic intoxication is an excellent example of how the presence of sickle cell trait can contribute to death. It is obviously true that if the patient were not intoxicated, and therefore somewhat anoxic, his cells would probably not sickle *in vivo*. Similarly, if his erythrocytes had not sickled, he probably would not have died from his acute alcoholic bout. During the past 5 years we have had several patients with sickle cell disease at this Institution in which the blood alcohol levels, taken both clinically and at necropsy were well below the accepted lethal range. However, only one of these patients had hemoglobin electrophoresis and could be included in this series.

While the presence of spontaneous hematuria (Conn[4], Crone *et al.*[5], Ende, Pizzolato and Ziskind[6], Greenberg, Kass and Castle[7], Henderson and Gray[8], Jenkins, Scott and Baird[9], Levin[10], MacIver, Went and Cruickshank[11]) and splenic infarction associated with high altitude flight (MacIver, Went and Cruickshank[11,12], McCormick[13]) is well recognized, the concept that sickle cell trait alone is, under certain conditions, a potentially dangerous, or even fatal, disease is generally not appreciated. Perhaps much of this controversy was due to the inability to accurately subdivide the sickle cell diseases prior to the introduction of alkali denaturation and paper electrophoresis. During the past several years, we have examined patients with sickle cell trait, as well as hemoglobin S-C disease and S-thalassemia, who were clinically diagnosed as having sickle cell anemia (S-S). For this reason, most of the work done prior to 1950, and a considerable proportion of that since, must be regarded as being inadequately studied.

The real problem involved is that sickle cell trait, unlike classical sickle cell anemia, does not produce marked clinical signs and symptoms under usual living conditions. However, add the stress of anoxia as from congestive heart failure, shock from any cause, or even a relatively mild respiratory tract infection, and fatal results may occur. The role of pH changes and

erythroconcentration, such as often occurs in congestive heart failure, on increased blood viscosity and sickling has been demonstrated *in vitro* by Greenberg, Kass and Castle[7].

Ende, Pizzolato and Ziskind[6] reported 3 patients with electrophoretically proven sickle cell trait in which they thought the clinical picture was due to the trait. These cases had involvement of the brain in one case, priapism in another, and jejunal infarction in the third. A fourth patient in which hemoglobin electrophoresis was not done, was necropsied and had pulmonary and renal infarcts and severe visceral congestion. Hemoglobin levels and the age tended to rule out sickle cell anemia, and the spleen size was against the diagnosis of S-C disease. This unproven case was the only one in which death occurred.

Tseng[23] reported 2 adults in which multiple visceral infarcts were found at necropsy. He thought that death in both of these patients was due to sickle cell trait. Unfortunately, electrophoresis to determine the true hemoglobin type was not performed. He recognized the possibility that hemoglobin S-C disease could have been present, and stressed the danger of any condition associated with hypoxia or shock.

More recently Jenkins, Scott and Baird[9] reported sudden death in 10 children whom they thought had sickle cell anemia. Again, electrophoretic proof of the hemoglobin type was not obtained so that sickle cell anemia is not proven in all their cases. In this connection, I have seen 6 infants with proven sickle cell trait with the same findings reported by Jenkins and coworkers.

A recent bulletin from the office of the medical examiner of the State of Virginia[18] states that "there is no longer room for doubt that such sickle states are potentially dangerous, especially in the presence of debilitating conditions." This publication stresses 3 general types of relationships of sicklemia to death: "(1) massive sickling in the presence of other diseases or pathology sufficient to cause death by itself (sickling might be only an incidental finding), (2) massive sickling in the presence of other disease insufficient to cause death by itself, and (3) massive sickling alone as an apparent cause of death." This excellent publication stresses the need for routine electrophoretic examination of the hemoglobin of Negro patients, and categorizes well the relationships of trait with death as seen in this study.

The question of the possibility that some of these cases represent S-thalassemia and not true sickle cell trait has been raised (Rucknagel[19]). This can be ruled out in most, if not all, of these cases by the findings of no pathological elevation of the fetal hemoglobin levels, adult hemoglobin (A) predominating over S in all cases, and no elevation of A_2 in those few cases in which starch block electrophoresis was done (Table 3).

MacIver and Went[11,12,24,25] stress that S-thalassemia may very closely resemble sickle cell anemia (S-S), but do not consider the differential diagnosis of S-thalassemia and sickle cell trait a difficult one. These workers discuss 3 possible pictures in S-thalassemia, namely, (1) complete suppression of hemoglobin A with the production of an S-S pattern on electrophoresis, (2) partial suppression of A with an S-A pattern in which S hemoglobin occurs in greater amounts than the normal pigment (A) and (3) a high fetal variant, which has been studied extensively in this area.

Brown and Ober[2], in a review of one aspect of the S-thalassemia problem, also stress splenomegaly as a common

feature, a finding absent in these 120 cases.

Finally, employing an immunologic technique (McCormick and Walker[14, 15]) differentiation of some of these cases from S-thalassemia is further supported.

Summary and Conclusions. Sickle cell trait can be reliably separated from hemoglobin S-C disease, S-thalassemia, and even sickle cell anemia only on the basis of careful hematologic and electrophoretic studies, even after necropsy has been performed. This is especially true in childhood.

The presence of definite antemortem visceral infarcts in 22 of 135 patients with proven sickle cell trait point out the dangers inherent in this vaso-occlusive disease. The well documented occurrence of hematuria and splenic infarction during high altitude flight in cases of sickle cell trait add weight to this necropsy study.

Sickle cell trait can, and does, cause death in and of itself, but more often, in association with other diseases or anoxemic states such as alcoholic intoxication, surgical anesthesia, and heart failure. For this reason, screening of all Negro patients is recommended, and measures taken to prevent anoxia in those with sickle cell disease.

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(Interlingua on page 295)

THYROTOXICOSIS ASSOCIATED WITH PERNICIOUS ANEMIA

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At the blood clinic run by the University Department of Medicine in the Glasgow Royal Infirmary it was noted that a surprising number of patients with pernicious anemia were, or had been, thyrotoxic. Of 100 consecutive patients attending as outpatients for follow-up, 2 were actively thyrotoxic, and another 5 patients had suffered from thyrotoxicosis in the past. In view of this striking incidence of the joint occurrence of the 2 diseases, it was decided to carry out the present investigation.

Each of 100 consecutive patients with pernicious anemia was interviewed and scored with the "thyrotoxicosis diagnostic index" evolved by Crooks, Murray and Wayne[6], which proved most useful in the present study. Significant features, positive and negative, in history and examination are given a numerical score based on the statistical analysis of the clinical features of a large group of thyrotoxic patients. For detailed discussion of the criteria and method of scoring the original article should be consulted. A score of +20 or higher indicates a clinical diagnosis of thyrotoxicosis.

The range of scores for the 100 patients with pernicious anemia was from −15 to +31 with a mean of −1.37. Two patients with active thyrotoxicosis scored +31 and +22 respectively. When these 2 patients were excluded the mean score for the remaining 98 was −1.94. The first 100 consecutive outpatients attending the blood clinic and a ward follow-up clinic who matched the pernicious anemia group by age and sex were also scored with the thyrotoxicosis diagnostic index. Of these 100 control patients, 37 had iron deficiency anemia, 16 were patients who had received treatment for iron deficiency anemia, 12 were attending for review after myocardial infarction, and 12 after miscellaneous conditions: peptic ulcer, bronchopneumonia, chronic bronchitis and emphysema, hypertension, refractory anemia, chronic myeloid leukemia, hemolytic anemia, and polycythemia. In the remaining 23 patients of the control group, most of whom suffered from mild anxiety state, no significant organic disease was found. The mean score for the control group was −5.38, with a range from −18 to +10. The difference in scores between the pernicious anemia patients and the control group is significant ('t' = 3.55 and $p<0.01$). The difference is also significant when the 2 frankly thyrotoxic patients are excluded ('t' = 3.23 and $p<0.01$). In the control group no thyrotoxic patients were found and none gave a history of thyrotoxicosis.

Further analysis of specific clinical features of the two groups reveals interesting divergences confined to physical signs. The thyroid gland was significantly enlarged in 30 of the pernicious anemia group, but was enlarged in only 17 of the control group. (X^2 = 4.69, 'p'<0.05). Hot hands were found in 39 of the patients with pernicious anemia and in 13 of the controls (X^2 = 17.6, 'p' <0.01). The hands were moist in 56 of the pernicious anemia patients and 32 of the controls. (X^2 = 11.69, 'p'<0.01). A resting pulse rate

of less than 80 per minute was found in 35 of the pernicious anemia group but in 63 of the control group (X^2 = 15.7, 'p'<0.01).

The records of all patients in whom pernicious anemia had been diagnosed between 1950 and May, 1959, were then reviewed. Before diagnosis of pernicious anemia was accepted the following features had to be present: macrocytic anemia with a megaloblastic bone marrow, histamine-fast achlorhydria, and reticulocyte response to therapy (initially liver extract, later cyanocobalamin - vitamin B_{12}). The group comprised 256 cases. Age and sex distribution is shown in Table 1; ages are of the last attendance at the clinic, or age at death, not age at diagnosis. Of the 256 patients, no fewer than 13 (11 women and 2 men) had suffered at one time or another from thyrotoxicosis, an incidence of 5.1%. It will be seen that in the total pernicious anemia population of 256 there were 76 men (30%), but of the 13 patients who also had thyrotoxicosis, only 2 were men (15%). Though the difference in sex incidence seems striking, it is not statistically significant (X^2 = 1.343, 'p'>0.1).

TABLE 1.—AGE AND SEX INCIDENCE OF 256 PATIENTS WITH PERNICIOUS ANEMIA

Age group	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100	Total
Number of women	2	7	19	39	45	59	8	1	180
Number of men	1	4	7	13	25	24	2	–	76

The diagnosis of hyperthyroidism was based on history, physical examination and radio-active iodine (I_{131}) tests. In some cases the basal metabolic rate was also determined. In the radio-active iodine tests a dose of 25 μc. of I_{131} was given. A combination of a thyroid gland uptake at 24 hours of more than 45% of the dose, and a protein-bound radio-active iodine level in the serum at 48 hours of more than 0.3% of the dose per liter, were the minimum requirements for a diagnosis of hyperthyroidism.

Clinical details of the 13 patients with both conditions are set out in Table 2. Comment on certain cases is necessary.

Case Reports. CASE 4. The diagnosis of pernicious anemia was made in 1941 in another hospital without a marrow biopsy. At that time the peripheral blood showed macrocytosis, anisocytosis and poikilocytosis of the red cells. Hemoglobin, which was 22% before treatment, rose to 74% in 4 weeks with liver extract. Peak reticulocyte response was 24%, and there was histamine-fast achlorhydria. The patient had a neurological syndrome suggestive of subacute combined degeneration which responded to the liver therapy.

CASE 6. This patient has refused to have radio-active iodine studies. She has been seen by several physicians who all concur in the diagnosis of thyrotoxicosis. Clinical features include excessive sweating, nervousness, recent increase in appetite, and palpitation. On examination there is evidence of weight loss. The patient is hyperkinetic, with a fine finger tremor and warm moist palms. The resting pulse rate is 110 per minute. There is a small firm goiter with a soft bruit. Scores with the diagnostic index have been consistently in the thyrotoxic range, varying from +22 to +26 on different occasions.

CASE 8. This patient was treated as an outpatient and the reticulocyte count was not followed. Response to therapy with vitamin B_{12} was good, with a rise in hemoglobin from 77% to 95%, and in red cell count from 2.8 millions to 4.25 millions per c.mm.

CASE 11. The diagnosis of thyrotoxicosis was readily made on clinical grounds in this patient. Thyrotoxicosis diagnostic index scores by 2 observers were +26 and +32. Border-

TABLE 2.—DETAILS OF 13 PATIENTS WITH PERNICIOUS ANEMIA AND THYROTOXICOSIS

Patient	*Age*	*Sex*	*Macrocytic anemia*	*Megaloblastic marrow*	*Reticulocyte response to therapy*	*Histamine fast achlorhydria*	*Clinically thyrotoxic*	*B.M.R.*	*Radio-active iodine tests*		*Date of diagnosis of pernicious anemia*	*Date of diagnosis of thyrotoxicosis*
									Gland uptake at 24 hours, % of dose	*Protein bound iodine at 48 hours, % of dose per liter*		
1	58	F	+	+	10.5	+	+	+63%	92	1.08	1953	1953
2	76	F	+	+	15.0	+	+	+45%	98	1.82	1949	1957
3	66	F	+	+	6.5	+	+	+28%	71	1.50	1955	1957
4	59	F	+	see text	24	+	+	+64%	80	1.86	1941	1950
5	64	F	+	+	29	+	+		91	1.12	1956	1956
6	58	F	+	+	19	+	+		see text	see text	1954	1957
7	29	F	+	+	14.5	+	+	+74%	80	3.92	1958	1956
8	37	F	+	+	see text	+	+		65	0.96	1957	1959
9	65	F	+	+	25	+	+	+32%	100	0.66	1947	1951
10	54	F	+	+	12.4	+	+		91	1.24	1954	1959
11	60	F	+	+	6.0	+	+		71	0.31	1956	1959
12	67	M	+	+	5.6	+	+		66	0.99	1952	1957
13	56	M	+	+	34	+	+		80	2.67	1953	1956

line results were obtained in radio-active iodine tests. However the total plasma radio-active iodine level 48 hours after the dose was 0.45% of the dose per liter, and this with the protein-bound radio-active level at 48 hours of 0.31% of the dose per liter gives a "conversion ratio" of 69%, further evidence to support the diagnosis of thyrotoxicosis (Clark[5]). The patient was given a therapeutic dose of radio-active iodine with subjective and objective improvement.

Only in one patient (Case 7) did thyrotoxicosis occur first. In 2 others (Cases 1 and 5) the onset of the 2 conditions was virtually simultaneous. The mean interval between the diagnosis of pernicious anemia and of thyrotoxicosis in the other 10 patients was 3.3 years, with a range of 21 months to 9 years.

For the remaining 156 patients not already compared with a control population, 156 control cases, matching the pernicious anemia cases by age and sex, were drawn from the general surgical wards of the Glasgow Royal Infirmary. For each patient in the control population a thyrotoxicosis diagnostic index score was determined, and in addition each patient was closely questioned about evidence of thyroid disease in the past. None of the control group was clinically thyrotoxic at the time of interview. One patient who had a subtotal thyroidectomy for thyrotoxicosis 2 months previously, gave a score with the diagnostic index of +8, and one other patient who had been treated surgically for thyrotoxicosis 5 years earlier gave a score of −3. The mean score for the total control group of 256 patients was −4.92, with a range of −18 to +10. The incidence of thyrotoxicosis, past or present, in the control population was thus 2 in 256, or 0.78%. This is significantly less than the incidence of thyrotoxicosis in the pernicious anemia population, that is, 5.1% ($X^2 = 8.06$, and $p<0.01$).

Discussion. The joint occurrence of pernicious anemia and thyrotoxicosis has been noted by many authors (Andrus and Wintrobe[1], Boenheim, Schwimmer and McGavack[4], Meulengracht[9], Murphy[10], Stenstam[11]). Wilkinson[17], in the largest reported series, found hyperthyroidism in 33 of 1600 patients with pernicious anemia. The combined incidence for all the series quoted above is 2.48%.

I have been unable to find any reliable studies on the incidence of thyrotoxicosis in the general population. Werner[14] writes "hyperthyroidism not being a reportable disease the prevalence of the disorder is difficult to establish." It is not a disease from which persons affected commonly die, so that one cannot use mortality statistics for judging its frequency.

There are of course disadvantages in the control population used in this study. Hospital admissions are inevitably selected samples of the general population and a different incidence of hyperthyroidism might have been found in a control group selected from the healthy population at large. A patient with 2 diseases may be more readily admitted to hospital than someone with one, leading to an over-representation in the hospital population of patients with apparently associated diseases. However, this factor is presumably common to the pernicious anemia group and the control group. A more important possibility is that the pernicious anemia population studied is not representative. The blood clinic at which all the pernicious anemia patients were followed is associated with the University Department of Medicine, which also has a special clinic for diseases of the thyroid, and it may be argued that a selected pernicious anemia population is seen. This possible objection is of doubtful validity however because in 10 of the 13 patients with the 2 disorders pernicious

anemia occurred first, the mean interval between the diagnosis of pernicious anemia and thyrotoxicosis being 3.3 years.

There has been considerable discussion as to a possible cause-effect relationship when pernicious anemia and thyrotoxicosis occur together. Achlorhydria following a 0.5 mg. dose of histamine is 4 times more common in hyperthyroid patients than in normal subjects, though soon after thyroidectomy the incidence returns to normal (Lerman and Means[7]); for this reason it has been suggested that in time untreated thyrotoxicosis may be followed by pernicious anemia (Andrus and Wintrobe[1]). It has also been suggested, without experimental support, that upset hepatic function due to thyrotoxicosis might be followed by pernicious anemia (Stenstam[11]). In animal experiments liver extract has no demonstrable effect on thyroid function (Meulengracht[9]), and thyroid extract, though increasing needs for vitamin B_{12}, has not produced anemia (Wallerstein[13]). A mild hypochromic anemia is common in severe hyperthyroidism and has been ascribed to the defect in gastric secretion (Whitby and Britton[15]).

Thyroxine seems to be a general marrow stimulant rather than a specific hematopoietic factor (Whitby and Britton[15]). Axelrod and Berman[2] found, in a study of the bone marrow in hyperthyroidism, that all elements of the bone marrow were hyperplastic. Erythropoiesis was normoblastic.

The possibility that upsets in gastric and liver function associated with thyrotoxicosis may lead to pernicious anemia cannot account for the findings in the present series in which 10 of the 13 patients were found to have pernicious anemia before evidence of thyrotoxicosis was detected, unless disordered gastric and liver function precede the clinical manifestations of thyrotoxicosis by some years.

A hyperthyroid tendency may have been unmasked by the high level of metabolic activity induced by vitamin B_{12} therapy. In a similar age-group not known to have pernicious anemia, levels of vitamin B_{12} and tissue metabolic activity may be lower.

The 100 control patients scored significantly lower in the thyrotoxicosis diagnostic index than the 100 patients with pernicious anemia. The difference between the 2 groups was largely due to the greater incidence of thyroid enlargement, hot moist hands, and rapid heart rate in the pernicious anemia patients. However as this control group was not a normal healthy population but comprised a heterogeneous population of patients with other blood diseases, anxiety states and so on, the significance of the differences found is difficult to assess. In the pernicious anemia group 30 patients had significant thyroid enlargement. Two of these patients were frankly thyrotoxic at the time of examination and it would be attractive to speculate that a number of the others who now show only thyroid enlargement may become thyrotoxic in due course.

Emotional stress is an important etiological factor in thyrotoxicosis; it has been suggested that as many as 80% of thyrotoxic patients are emotionally disturbed at the time of onset (Lidz[8]). It may be wondered if the mental upset associated with the diagnosis of pernicious anemia, an 'incurable' disease requiring regular supervision and lifetime treatment, may have resulted in the increased incidence of thyrotoxicosis.

There is no doubt that heredity is an important factor in the etiology of pernicious anemia (Whitby and Britton[16]), and there is also evidence for a familial incidence of diffuse toxic goiter (Bar-

tels[3]); there may perhaps on occasion be a common pathway of inheritance.

In the present study a higher incidence of thyrotoxicosis complicating pernicious anemia has been found than ever previously reported. The incidence of thyrotoxicosis is so high compared with that found in a control population that it is difficult to agree with Wilkinson[18] and other authors (Andrus and Wintrobe[1], Boenheim, Schwimmer and McGavack[4]) in ascribing the association to chance. It is most interesting that a striking association between classical Addisonian pernicious anemia and myxedema has recently been reported by Tudhope[12] who found 13 cases of pernicious anemia in 116 patients with myxedema. He concluded that deficiency of thyroid hormone did not by itself lead to pernicious anemia; the reason for the association of the two conditions was not clear.

No answer emerges from this study as to what factors may be involved in the association of thyrotoxicosis with pernicious anemia. The only firm conclusion is that the incidence reported here, that is, 13 cases of thyrotoxicosis in 256 cases of pernicious anemia, seems to be much higher than can be explained on a basis of coincidence. The problem, which involves the hematologist and the endocrinologist requires further study. It is hoped that the present paper will help to stimulate interest in the problem.

Summary. Of 256 cases of pernicious anemia, 10 developed thyrotoxicosis at a mean interval of 3.3 years after the diagnosis of pernicious anemia. In 2 patients, onset of the 2 conditions was virtually simultaneous. One patient developed pernicious anemia after treatment of thyrotoxicosis. In a control population of 256 patients selected from a blood clinic and general surgical wards, 2 patients gave a history of thyrotoxicosis. The difference in incidence of thyrotoxicosis between the pernicious anemia group and the control group is significant. One hundred patients with pernicious anemia were assessed with the 'thyrotoxicosis diagnostic index.' They gave a statistically significantly higher score than 100 control patients, though the real meaning of this finding is difficult to assess.

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SUMMARIO IN INTERLINGUA

Thyrotoxicosis, Associate con Anemia Perniciose

Inter 256 patientes con anemia perniciose, 10 disveloppava thyrotoxicosis post un intervallo medie de 3,3 annos a partir del diagnose de anemia perniciose. In 2 patientes, le declaration del 2 conditiones esseva virtualmente simultanee. Un patiente disveloppava anemia perniciose post esser tractate pro thyrotoxicosis. In un population de controlo de 256 patientes seligite ab un clinica hematologic e divisiones de chirurgia general, 2 reportava un anamnese de thyrotoxicosis. Le differentia in le incidentia de thyrotoxicosis inter le gruppo con anemia perniciose e le gruppo de controlo es significative. Cento patientes con anemia perniciose esseva evalutate con respecto al "indice diagnostic de thyrotoxicosis." Illes produceva valores plus alte per un differentia de signification statistic que 100 patientes de controlo. Tamen, le ver importantia de iste constatation es difficile a determinar.

SEVERE REACTIVE PULMONARY HYPERTENSION SECONDARY TO AORTIC STENOSIS

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Varying degrees of pulmonary hypertension and pulmonary vascular changes have been described in patients with mitral stenosis (Heath and Whitaker[5], Wood[11]). In addition, structural changes in the pulmonary vessels have been described in chronic left ventricular failure due to a variety of causes (Smith, Burchell and Edwards[10]). During the course of evaluation of candidates for cardiac surgery, it has become apparent that such changes can be associated with aortic stenosis, and that these changes can be important.

The purpose of this paper is to present 2 cases in which such changes occurred and to discuss the various factors involved in the pathogenesis of this pulmonary vascular reaction.

Case Reports. case 1. A 47-year-old white man was admitted for evaluation for cardiac surgery. Although he had frequent sore throats during childhood, he did not recall ever having had either swollen, painful joints or any other symptoms of rheumatic fever. He served during World War II with the Marine Corps, and was discharged from the service without any disability in 1945. In 1957, during a physical examination for an upper respiratory infection, a heart murmur was discovered. Since then, he had developed progressively severe and incapacitating paroxysmal nocturnal dyspnea, dyspnea on exertion, and easy fatigability. During the 6 months prior to admission, he had numerous fainting spells and experienced exertional chest pain which was relieved by rest.

Physical examination. The patient was a well developed, well nourished, white man. Pulse: 90 per minute. Blood pressure: Right arm, 120/80 mm. Hg. Left arm, 107/68 mm. Hg. Neck veins were distended but not otherwise remarkable. Examination of the chest revealed generalized wheezing and numerous sibilant rales bilaterally. The heart was enlarged to the left on percussion. A left ventricular thrust was noted in the sixth intercostal space midway between the midclavicular line and the anterior axillary line. No thrills or shocks were palpable. Auscultation revealed a harsh grade III (on the basis of VI) obstructive type, turbulent systolic murmur, loudest at the first and second right intercostal spaces with radiation into the neck as well as across the entire precordium. The murmur was also heard in the back. The aortic second sound was diminished. The pulmonic second sound was accentuated though not particularly reduplicated. A grade II blowing, decrescendo, early diastolic murmur was heard along the left sternal border. The liver was palpable two finger-breadths below the costal margin and tender. The remainder of the general examination was negative. Decholin circulation time was 25 seconds. Venous pressure was 138 mm.

Electrocardiogram. Revealed changes consistent with left ventricular hypertrophy and digitalis effect.

Cardiac roentgenograms and chest fluoroscopy. The diaphragms moved well. The lung vascularity was increased. The heart was generally enlarged. The left ventricle was particularly prominent, as were the left atrium and ascending aorta. The right atrium, right ventricle and main pulmonary artery were of normal size. Calcification was present in the aortic valve.

Cardiac catheterization. Right heart catheterization was performed through the right basilic vein. The left heart catheterization was performed paravertebrally by inserting a needle into the left atrium. A systolic gradient across the aortic valve of 63 mm. of mercury was noted. Main pulmonary artery pressure was 86/40 mm. Hg. The impression of the cardio-pulmonary physiologists was that these data indicated severe pulmonary hypertension and aortic stenosis (Table 1).

Admission diagnosis. Severe calcific aortic stenosis, mild aortic insufficiency, mild congestive heart failure, severe reactive pulmonary hypertension.

TABLE 1.—CARDIAC CATHETERIZATION

	Normal	*Case 1*	*Case 2*
Body surface area (m^2 BS)	—	1.96	1.64
Oxygen consumption ml./min./m^2	135	135	264
Arteriovenous oxygen difference (Vol.%)	4.2	7	3.57
Cardiac output (L./min.)	3.1–4.20	3.9	7.4
Cardiac index L./min./m^2 BS	3.2	2.0	4.5
Pressure in mm. Hg			
Right Atrium	4/2	18/6	15.5/3.5
Right ventricle	25/0	86/4/19	97/4
Main pulmonary artery	25/10	86/40	97/33
Pulmonary wedge artery	5–10	33	42
Left ventricle	135/10	175/12/30	200/10
Aorta	135/70	112/65	147/67
Brachial artery	135/70	112/65	147/67
Blood Oxygen Content in Vol.%			
Right atrium	—	—	11.50
Right ventricle	—	—	12.35
Main pulmonary artery	—	8.69	12.34
Left ventricle	—	—	—
Aorta	—	15.66	15.91
Brachial artery	—	15.66	15.91
Blood Oxygen Saturation in %			
Right atrium	—	—	62.5
Right ventricle	—	—	63.3
Main pulmonary artery	—	46.4	63.9
Left ventricle	—	—	—
Aorta	—	83.6	86.4
Brachial artery	—	83.6	86.4
Total Pulmonary Resistance dynes/sec./cm^{-5}	150–250	1150	582
Pulmonary Arteriolar Resistance dynes/sec./cm^{-5}	50–150	1614	304
Pulmonary Flow at Rest L./Min./m^2	3.1–4L	3.9	7.4

Course. All routine laboratory tests were within normal limits. The patient was given digitalis, diuretics, and a low-salt diet. On this regimen, he lost 14 pounds. He was scheduled for aortic commissurotomy, but he died suddenly 4 days before the scheduled surgery.

Necropsy findings (Fig. 1). 1) Chronic rheumatic endocarditis; 2) calcific aortic stenosis; 3) mild mitral valve fibrosis without significant stenosis; 4) concentric hypertrophy

Fig. 1

of left ventricle, severe (heart weight, 565 gm.); 5) chronic passive congestion of lungs and spleen; 6) mild to moderate local arteriosclerosis of the lungs; 7) Hydrothorax, right chest (1700 ml.); 8) dilatation of aorta.

Microscopic examination of lungs. The large arteries generally showed minimal fibrous thickening of the intima, with the presence of a few lymphocytes and mononuclear cells. Many of the small pulmonary vessels were definitely thickened, possessing loose laminated fibrous intima, a rather thin media, and narrowed lumina. There was moderate, fibrous, lobular, interstitial thickening of the alveolar septae suggestive of small areas of healed infarction.

COMMENT. Though the patient was in mild congestive failure at the time of catheterization, the observed pulmonary hypertension was out of proportion to that seen secondary to passive "back pressure" associated with myo-

cardial decompensation. The pressure of 86 mm. systolic in the pulmonary artery was probably secondary to a reaction within the pulmonary vascular structures.

CASE 2. A 54-year-old white woman was admitted to National Jewish Hospital on August 22, 1958. At age 4, she developed an illness characterized by swollen, red, warm joints accompanied by fever. During childhood she had had frequent sore throats. There had been 7 pregnancies with normal deliveries. In 1946, at age 42, she was first told that she had a heart murmur. During the 5-year period, and particularly the last 3 years, prior to admission to this hospital, she had noted progressive exertional dyspnea, blood streaked sputum, and repeated episodes of "bubbling" in her chest. For a year prior to admission, she had marked perspiration accompanied by a burning sensation in her anterior precordium radiating to the neck and both shoulders occurring during exertion.

Physical examination. The patient was a well developed, slightly obese white woman. Pulse: 76 per minute. Blood pressure: Right arm, 130/98 mm. Hg. Left arm, 126/86 mm. Hg. The general examination was essentially negative. The pulse was irregularly irregular. The heart was enlarged to the left. Examination of the neck revealed prominent A-waves. A systolic thrill with nuchal radiation was felt at the base of the heart. A left ventricular thrust was noted. Auscultation revealed a harsh, obstructive type, systolic murmur, grade IV in intensity (on grade VI basis), accentuated in midsystole, and accompanied by a faint, grade I, decrescendo, blowing diastolic murmur. The systolic murmur was loudest at the second right interspace. The diastolic murmur was of maximum intensity in the third and fourth left intercostal spaces. There was a blowing, grade II, high pitched apical systolic murmur with radiation to the left axilla. The aortic second sound was not heard, and the mitral first sound was muffled.

Electrocardiogram. Changes were noted which were consistent with incomplete left bundle branch block, left ventricular hypertrophy, and digitalis effect.

Cardiac fluoroscopy. There was marked enlargement of the heart, with the left ventricular shadow being particularly prominent. A barium swallow revealed also enlargement of the left atrium. Calcification of the aortic valve was demonstrated by tomography.

Admission diagnosis. Severe calcific aortic stenosis, mild aortic insufficiency, mild mitral insufficency, and severe pulmonary hypertension.

Cardiac catheterization. Right heart catheterization was performed by standard techniques. Left ventricular pressures were obtained by direct puncture through the anterior precordial route. The results are summarized in Table 1. A 53 mm. gradient was present in systole across the aortic valve. The main pulmonary artery pressure was 97/33 mm. Hg. Thus severe aortic stenosis accompanied by severe pulmonary hypertension was evident.

Course. The patient underwent aortic commissurotomy on September 5, 1958, with the use of the Brock dilator inserted through the left ventricular wall into the aortic valve area. During the procedure, she developed ventricular fibrillation which reverted to normal sinus rhythm after massage. Following surgery, blood pressure was maintained with norepinephrine by intravenous drip. Eighteen hours after surgery, her blood pressure suddenly became unobtainable. She became cyanotic, and her heart stopped beating.

Necropsy findings (Fig. 2). 1) Chronic rheumatic endocarditis; 2) calcific aortic valvular stenosis, severe; 3) mitral valvulitis, mild; 4) tricuspid valvulitis, mild; 5) hypertrophy of left ventricle, severe (total heart weight, 710 gm.); 6) chronic passive hyperemia of lungs and spleen; 7) mild acute fibrinous pericarditis; 8) edema of lungs.

Microscopic examination of lungs. There was a moderate diffuse fibrous intimal thickening of the small pulmonary arteries with small patches of foam cells and a scattering of lymphocytes and polymorphs visible in the arterial wall. The lung parenchyma was hyperemic, and the alveoli contained numerous loosely scattered brown pigmented phagocytes and unpigmented mononuclear cells.

COMMENT. A pulmonary artery pressure of 97/33 mm. Hg was out of proportion to what one might expect from uncomplicated "back pressure" secondary to passive congestion of the lungs. The mild mitral valvulitis present in this patient appeared to be of little consequence. The severe aortic stenosis was the only significant valvular deformity.

Discussion. Pulmonary hypertension has been classified by Wood[11] as follows: 1) Passive. 2) Hyperkinetic. 3) Vaso-occlusive. 4) Reactive. Passive

Fig. 2

pulmonary hypertension follows any condition that raises the pulmonary venous pressure, that is, left ventricular failure, mitral valve disease, cor triatriatum, multiple pulmonary venous thrombosis or stenosis, or myxoma of the left ventricle. The pulmonary arterial pressure in such instances is around 50/25 mm. Hg.

Hyperkinetic pulmonary hypertension is the reaction usually associated with an increased flow. However, until maximum vasodilatation has been achieved, an increased flow such as in the secundum type of atrial septal defect does not usually result in pulmonary hypertension. An increased blood flow up to three times normal can be counter-balanced by reductions of resistance to one-third normal.

Vaso-occlusive pulmonary hypertension follows the closing of the vascular pathways through the lung at the pulmonary capillary, arteriolar, or arterial level and this may be secondary to: 1) Extrinsic or intrinsic occlusive phenomena. 2) Structural diseases within the vessels themselves, such as accompanies schistosomiasis, lupus erythematosus disseminatus and perhaps other forms of arteritis. 3) A vaso-constrictive reaction to pulmonary hypertension itself. This reaction is believed to be a normal physiologic response of the muscular pulmonary arteries to a rise of tension within them.

Although the responsible factors are not as yet clearly defined in the cases presented, the degree of pulmonary hypertension appears to place them in the reactive as opposed to the passive variety. Originally it was believed that such anatomic pulmonary vascular changes were associated with left heart disease only when mitral stenosis was present. Smith, Burchell and Edwards[10], however, observed similar changes in a case of left ventricular failure secondary to calcific aortic stenosis. Heath, Cox and Harris-Jones[4] also reported on the association between myocardial fibrosis and pulmonary arterial hypertension.

In 1927 the possibility that pressoreceptors could be responsible for the reactive changes in the pulmonary vasculature was initially given some support when De Burgh Daly, and Verney[3] described pressoreceptors in the left side of the heart whose reflex arc depends on an intact vagus nerve. Aviado *et al.*[1], while studying a perfused right dog heart, demonstrated a similar reflex mechanism.

A humoral mechanism was first described by Rossi and Zamboni[8] when they induced experimental pulmonary atherosclerosis in the small arteries and arterioles of rabbits with the injection of serotonin (5-hydroxytryptamine). Nahas[7] introduced serotonin into the left atrium of an intact dog producing a rise in the pulmonary artery pressure without a similar rise in the aorta or pulmonary veins. Rudolph and Paul[9] observed that if serotonin creatinine sulfate was administered at the rate of 150 μg. per kg. per minute, the pulmonary artery pressure was maximally increased to 300% over control levels. Systemic resistance, however, was decreased by 55%. Endogenous sources of serotonin in congenital and acquired heart lesions could be the disruption of platelets caused by the turbulence of blood in the region of the cardiac defect (Comroe *et al.*[2]).

Kuida *et al.*[6] attributed the pulmonary vascular changes to an endotoxin and they noted that the endotoxin from *E. coli* when administered to cats caused severe pulmonary hypertension which within a few days resulted in right heart failure and death. They speculated that the endotoxin did not act directly upon the smooth muscle of the pulmonary vasculature but rather through the liberation of some agent or agents into the blood stream.

Thus it would seem reasonable to conclude that aortic stenosis can be added to those left heart abnormalities eventuating in severe pulmonary arterial hypertension. This is of clinical importance since the presence of this vascular lesion does add significantly to the risk of surgery, and the sudden deaths which occur in patients with stenotic lesions of the aorta may involve mechanisms similar to those eventuating in a similarly sudden demise in cases of primary pulmonary hypertension.

Summary. Two cases of aortic stenosis associated with severe pulmonary arterial hypertension are presented. In

both instances the diagnosis was confirmed by cardiac catheterization and also, in one case, by postmortem examination. Possible mechanisms and implications of the associated vascular reaction are discussed.

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SUMMARIO IN INTERLINGUA

Sever Reactive Hypertension Pulmonar, Secundari a Stenosis Aortic

Es presentate 2 casos de stenosis aortic associate con sever hypertension pulmono-arterial. In ambe casos le diagnose esseva confirmate per catheterismo cardiac e, in un, etiam necropticamente. Le mechanismos possibile e le signification inherente del associate reaction vascular es discutite.

SERUM ENZYMES IN DISEASE
VII. SIGNIFICANCE OF ABNORMAL SERUM ENZYME LEVELS IN CARDIAC FAILURE*

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Since the original report of LaDue, Wroblewski and Karmen[12] of the occurrence of elevated serum levels of glutamic oxaloacetic transaminase (GOT) in patients with acute myocardial infarction, determination of this serum enzyme has become a "routine" laboratory procedure. In early reports it was suggested that heart failure without myocardial infarction would not produce elevated serum GOT levels (Merrill, Lemley-Stone and Meneely[17], LaDue and Wroblewski[11], Kattus *et al.*[9], LaDue, Wroblewski and Karmen[12], Steinberg and Ostrow[26]). More recent studies (Denney *et al*[3], Shabetal, Iglauer and Anderson[23], Dutoit[5], Fragge, Kopel and Iglauer[8]), however, have revealed that in heart failure a low (10 to 25%), but appreciable incidence of serum GOT values may be observed, even in the absence of infarction or coronary insufficiency. It has been suggested that these elevations of serum GOT levels are reflections of hepatic congestion and centrilobular hepatic necrosis in patients with heart failure (Fragge, Kopel and Iglauer[8]).

In the present study, the levels of GOT and of 8 other serum enzymes have been determined and tests of hepatic function have been performed in a large group of patients with heart failure, in an attempt to clarify mechanisms responsible for elevated serum enzyme levels in this condition.

Materials and Methods. Two-hundred and twenty-four patients were studied during an acute episode of cardiac decompensation. The patients were separated into those with predominantly right, left or "combined" heart failure on the basis of venous pressure determinations and clinical features.

Patients with recent myocardial infarction, hypotension or shock, pulmonary infarction, cerebral vascular accidents, history of alcoholism, any type of intrinsic hepatic disease, blood dyscrasias, malignancies, pancreatitis or muscle disease were excluded from this study.

The patients were derived from two groups: the first, from a Veterans Hospital, were largely males; the other, from a private hospital, included males and females.

The enzyme determinations were performed according to standard methods with minor

*This study was made possible by a grant from the Chicago Heart Association.

modifications. *Phosphohexose isomerase (PHI)* was assayed by Bodansky's method[2]. The micromethod of Lowry[13] was modified to permit the determination of *aldolase* activity in 0.1 ml. of serum (Weinstein, Muniak and Zimmerman[30]). For *lactic dehydrogenase (LD)* the method of Neilands[19] as modified by Zimmerman and Weinstein[35], was performed. The method of Ochoa[20] was used for the determination of *malic dehydrogenase (MD)*. For the measurement of *isocitric dehydrogenase (ICD)* the method described by Wolfson and Williams-Ashman[34] was employed. The two *transaminases, glutamic oxaloacetic transaminase (GOT)* and *glutamic pyruvic transaminase (GPT)* were measured by the modification of Reitman and Frankel[22] of the method of Umbreit[27,28]. *Glutathione reductase (GR)* levels were determined by the method of Racker[21] as modified by West, Rony and Zimmerman[32]. In this method the disappearance of $TPNH_2$ in the presence of oxidized glutathione and serum is measured spectrophotometrically at 340 mμ.

The tests of hepatic function included the bromsulfalein (BSP) excretion according to the modification of Mateer (using 5 mg. per kg. and determining the degree of dye retention in 45 minutes)[16], the thymol turbidity by the procedure of Maclagen[14] as modified by Shank and Hoagland[24], the cephalin flocculation by the method of Hanger, as modified by Neefe and Reinhold[18], the alkaline phosphatase by the Bodansky method[1] and the serum bilirubin by the Malloy and Evelyn method[15]. The level of the total serum proteins and the albumin and globulin fractions was determined by the Weichselbaum modification[29] of the Kingsley biuret method[10].

The upper limits of normal, in our laboratory, of the serum enzymes and of the tests of hepatic function are presented in Table 1.

Results. Elevated levels of GOT were observed in 12% of the entire group of patients (Table 2). The increased values occurred as often in patients with left heart failure as in those with right heart failure (Fig. 1). Increased levels of GPT occurred approximately as often as those of GOT in patients with right heart failure, whether alone or combined with left heart failure. In patients with "pure" left heart failure, however, only 1 of 39 individuals showed an elevated value for the GPT (Fig. 1).

A much higher incidence of abnormal levels of aldolase, PHI, LD and MD was observed in patients with heart failure (Table 2). There was no significant difference between patients with right and left heart failure in the case of all of these enzymes, except for the malic dehydrogenase. The level of the latter serum enzyme was abnormal in only 1 of 12 patients with right heart failure, whereas an abnormal value

TABLE 1.—UPPER LIMITS OF NORMAL FOR TESTS PERFORMED

Determination	
Glutamic Oxaloacetic Transaminase (GOT)	40 units
Glutamic Pyruvic Transaminase (GPT)	38 units
Phosphohexose Isomerase (PHI)	95 mμM F-6-P/ml./min.
Aldolase (ALD)	3.8 mμM F-1, 6-P/ml./min.
Lactic Dehydrogenase (LD)	270 μM DPNH/100 ml./30 min.
Malic Dehydrogenase (MD)	75.0 mμM DPN/ml./min.
Isocitric Dehydrogenase (ICD)	10.0 mμM DPN/ml./min.
Glutathione Reductase (GR)	75 units
Alkaline Phosphatase (AP)	40 units
Bromsulphalein (BSP)	5.0%
Serum Albumin	<3.6 gm.%
Serum Globulin	3.5 gm.%
Thymol Turbidity (T.T.)	4.0 units
Cephalin Flocculation (C.F.)	2(+) in 24 hrs.
Total Bilirubin	1.0 mg. per 100 ml.

TABLE 2.—SUMMARY OF RESULTS

Determination	*Number of Patients*	*Number Abnormal*	*% Abnormal*
BSP	110	107	97%
Bilirubin	130	56	43%
Albumin	199	108	54%
Globulin	202	17	8%
Thymol Turbidity	123	19	15%
Cephalin Flocculation	63	6	9%
Alkaline Phosphatase	154	100	65%
GOT	224	28	12%
GPT	169	18	11%
PHI	54	34	63%
ALD	69	33	48%
LD	181	70	39%
MD	44	13	30%
ICD	37	5	14%
GR	27	2	8%

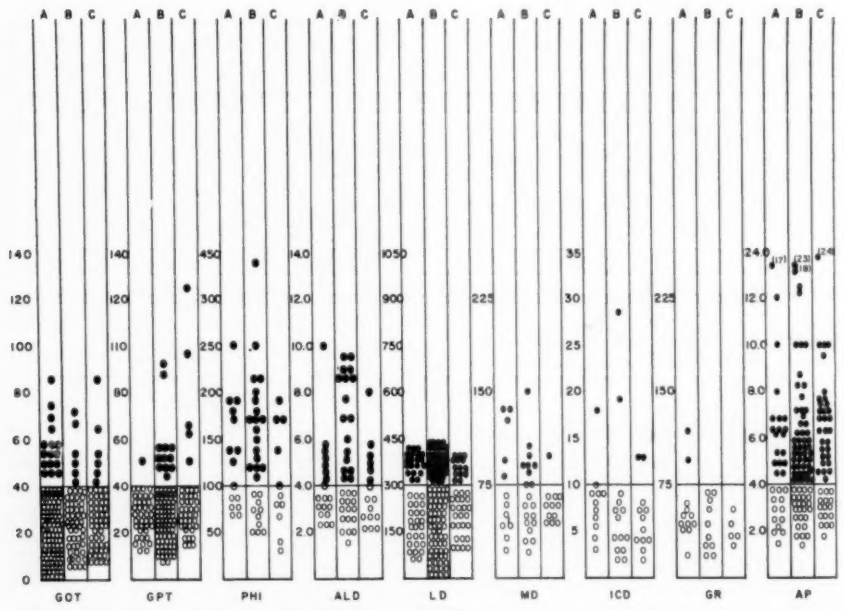

Fig. 1.—Scattergram showing highest enzyme values determined in patients with left heart failure (column "A"), right heart failure (column "C") and "combined" heart failure (column "B"). The black circles represent abnormal levels, the white circles normal values.

was observed in 42% of patients with left heart failure (Fig. 1). Levels of ICD and GR were abnormal in a small proportion of patients with left heart failure. None of the small group of patients with right failure in whom GR levels were determined had elevated values. Values of ICD were abnormal in approximately the same low incidence of right (17%), left (9%), or combined heart failure (14%). Abnormal alkaline phosphatase values were recorded in approximately 65% of the patients with heart failure, irrespective of the type (Fig. 1).

HEPATIC FUNCTION TESTS. Abnormal BSP retention was observed in 97% of the patients with heart failure (Table 2) (Fig. 2). Nevertheless, as shown in Fig. 3, there was a direct correlation between the degree of BSP retention and the level of peripheral venous pressure ($r = +0.64$, $P<0.001$). Hyperbilirubinemia was observed in a fourth of the patients with left heart failure and approximately half of those with combined or right heart failure (Fig. 2). Abnormal results with thymol turbidity and cephalin flocculation tests were found in less than one-fourth of the patients with right or left heart failure. Mild hypoalbuminemia was observed in approximately half of the patients with heart failure irrespective of the type (Table 2) (Fig. 2). Hyperglobulinemia of modest degree occurred more frequently in patients with right heart failure (17%) and combined failure (12%) than in patients with left heart failure (6%).

Attempts to correlate the level of the transaminases with the level of peripheral venous pressure revealed two apparent populations (Fig. 4).

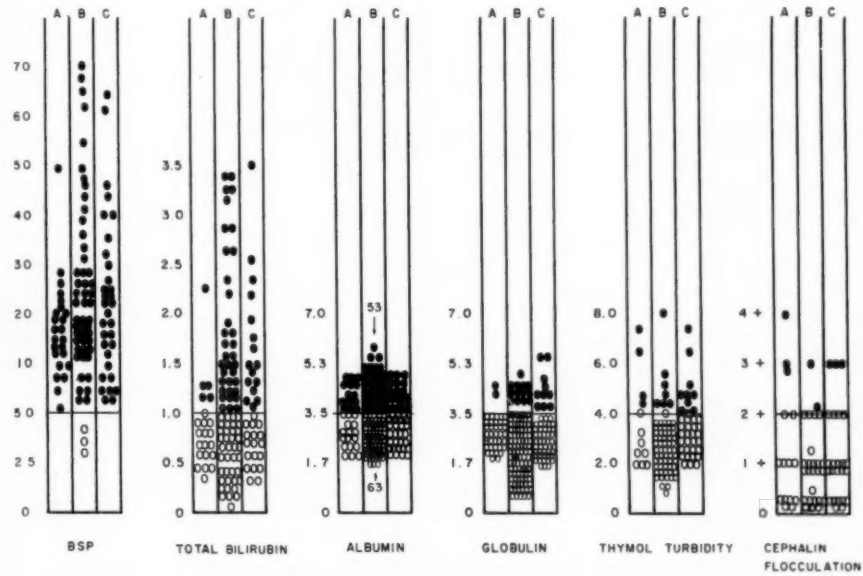

Fig. 2.—Scattergram showing highest values of the hepatic function tests determined in patients with left heart failure (column "A"), right heart failure (column "C"), and "combined" heart failure (column "B"). The black circles represent abnormal levels, the white circles normal values.

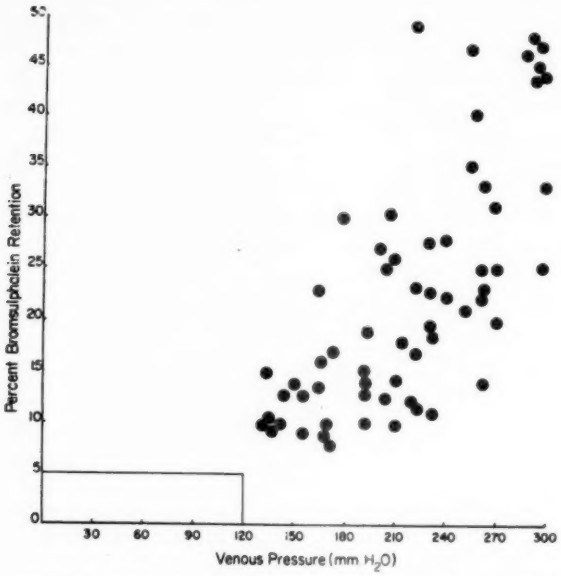

Fig. 3.—Scattergram showing per cent of BSP retention plotted against the level of peripheral venous pressure simultaneously determined in each patient. As can be seen, there is a direct correlation between these two tests ($r = +0.64$, $P<0.001$).

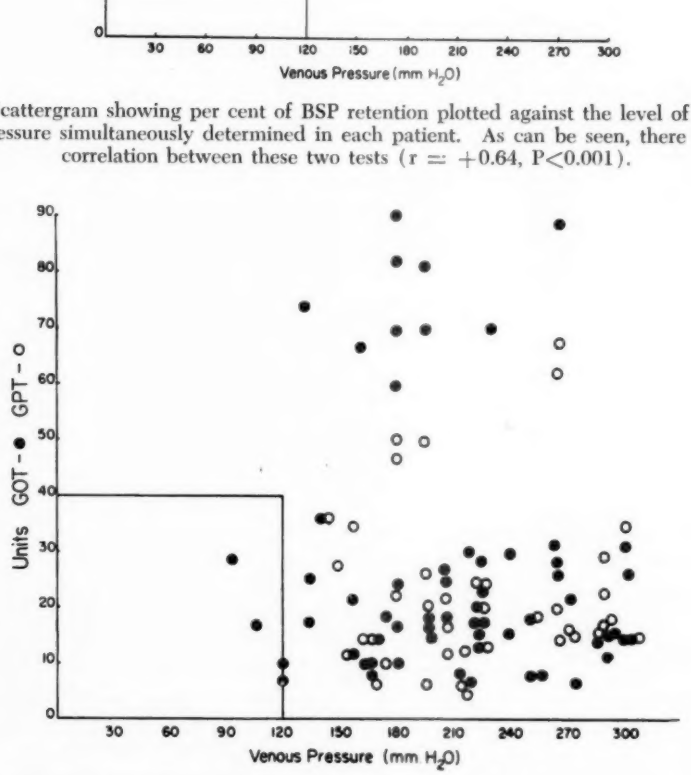

Fig. 4.—Scattergram showing levels of GOT and GPT plotted against venous pressure values simultaneously determined. A positive correlation appeared to exist only when the transaminases were elevated.

Inspection of Fig. 4 reveals that the correlation between the level of venous pressure and the level of either transaminase was significant for the patients with elevated transaminase values, but for the remaining patients no correlation with the venous pressure level was apparent.

Discussion. The incidence of hepatic dysfunction in this group of patients is comparable to that previously reported by others (Evans *et al.*[6], Felder, Mund and Parker[7], Sherlock[25], White *et al.*[33]). The degree of impairment of BSP excretion and of serum bilirubin elevation correlated well with the type of the heart failure and the degree of venous hypertension. These two parameters have been considered to reflect hepatic venostasis and congestion, although even in patients with "pure" left heart failure impaired BSP excretion may be observed. It should be kept in mind, however, that the attempts to separate right from left heart failure may be extremely difficult, particularly without the benefit of determination of end-diastolic pressure in the right ventricle.

Elevations of GOT were observed in 12% of this group of patients. It would appear from the data presented that to attribute GOT elevations to hepatic congestion or centrilobular necrosis would be an over-simplification, since elevated values were obtained in left as well as right heart failure. In patients with elevated levels, however, a direct correlation with the height of venous pressure was observed. It may be inferred that in addition to venous hypertension, some other factor may be contributing to GOT elevations.

Elevated levels of GPT, however, appear to bear a more direct relationship to the type of heart failure. In patients with right heart failure GPT elevations were present approximately as often as were those of GOT. In patients with left heart failure GPT levels were normal.

Elevated levels of other enzymes, cannot be readily attributed to hepatic involvement. The three glycolytic enzymes (LD, aldolase and PHI) were elevated in 40 to 60% of patients with heart failure, irrespective of the type. This would suggest a relationship to some factor other than hepatic congestion. This impression is supported by observations in patients with intrinsic hepatic disease where LD elevations are less often seen and less marked than those of GOT or GPT elevations (West and Zimmerman[32]). While the basis for the elevation of the glycolytic enzymes is not clear, it is intriguing to consider the possibility that tissue anoxia characteristic of heart failure may lead to increased tissue content of glycolytic enzymes. Increased release of such intracellular enzymes into the serum may result from altered cell wall permeability incident to the heart failure. It is of interest that we have observed moderate elevations of these enzymes in more than half of the patients with acute episodes of bronchial asthma with severe anoxia.

The elevated levels of malic dehydrogenase, an enzyme presumably involved in citric acid cycle activity, but also in other metabolic pathways in the cell, permit no ready explanation. There was a higher incidence of elevated values for this enzyme in patients with left heart failure than in those with right heart failure. Further studies are required to clarify the meaning of these results. The low incidence of isocitric dehydrogenase elevations in patients with right or left heart failure and the GR elevation in patients with left heart failure are of interest, but again, there is no apparent explanation for this. While it might have been assumed that alkaline phosphatase elevations in patients with heart failure

represent hepatic dysfunction the results of the present study do not quite conform to this suggestion. The incidence of alkaline phosphatase elevation was surprisingly high (65%) with no difference between the patients with right and left heart failure. In a few patients levels as high as 15 to 20 Bodansky units were observed.

It is of interest that all of the enzymes studied are "soluble" enzymes, that is they are located in the cytoplasm rather than in the mitochondria of the cell (Dixon and Webb[4]). Alteration of cell permeability incident to heart failure may contribute to or be responsible for the leaching of soluble enzymes from the cell.

While the mechanisms for serum enzyme elevation in heart failure are obscure, it may be concluded that it occurs frequently. It should be kept in mind that elevated levels of GOT in a patient with heart failure do not necessarily reflect myocardial infarction, unless other evidence is quite convincing. It may be inferred that the GOT elevation represents heart failure rather than infarction, if the GPT level is also elevated and, particularly, if it is elevated more than the GOT value. Elevations of LD and other glycolytic enzymes are seen with such frequency in heart failure that they may be considered characteristic. While there are other more conventional parameters of severity of the heart failure which may be applied, serum enzyme abnormalities as well as the measures of hepatic function may be used with profit to follow the course of patients with heart failure.

Summary. Two-hundred and twenty-four hospitalized patients during an acute episode of cardiac decompensation were studied. Patients with hypotension, shock, recent myocardial infarction, pulmonary infarction, cerebral vascular accidents, history of alcoholism and any type of intrinsic hepatic disease were excluded from this study.

Levels of glutamic oxaloacetic transaminase and glutamic pyruvic transaminase were abnormal in 12% and 11% respectively; of lactic acid dehydrogenase in 39%; of aldolase in 48%; of phosphohexose isomerase in 63%; of isocitric dehydrogenase in 14%; of malic hehydrogenase in 30%, and of glutathione reductase in 8%. Abnormal levels of alkaline phosphatase were found in 65%.

Almost all (97%) of the patients had abnormal bromsulfalein retention values. Hyperbilirubinemia was present in 43%. Thymol turbidity and cephalin flocculation were abnormal in 15% and 9%, respectively. The serum albumin level was depressed in 54% of patients, while hyperglobulinemia was present in 8%.

While the venous pressures positively correlated with the degree of bromsulfalein retention ($r = +0.64$), the former correlated with the transaminase levels only when the latter were abnormal.

The levels of glutamic pyruvic transaminase were significantly higher in patients with right rather than those with left heart failure, suggesting a relationship to hepatic congestion. The levels of the other enzymes were equally elevated in right and left heart failure or were even higher in patients with predominately left heart failure. This latter observation suggests a relationship to metabolic abnormalities more generalized than only the hepatic congestion of heart failure.

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SUMMARIO IN INTERLINGUA

Enzymas Seral in Morbo
VII. Signification de Anormal Nivellos de Enzymas Seral in **Insufficientia** Cardiac

Esseva studiate 224 hospitalisatos durante episodios acute de decompensation cardiac. Esseva excludite ab le studio patientes con hypotension, choc, infarcimento myocardial recente, infarcimento pulmonar, accidentes cerebro-vascular, anamnese de alcoholismo, e omne genere de morbo hepatic intrinsec.

Le nivellos de transaminase glutamic-oxaloacetic e de transaminase glutamic-pyruvic del sero esseva anormal in 12, respectivemente 11% del casos. Pro dishydrogenase de acido lactic, le nivellos esseva anormal in 39%; pro aldolase in 48; pro isomerase de phosphohexosa in 63; pro dishydrogenase isocitric in 14;

pro dishydrogenase malic in 30; e pro reductase de glutathiona in 8. Nivellos anormal de phosphatase alcalin esseva trovate in 65%.

Quasi omne le patientes (97%) habeva valores anormal pro le retention de bromsulfaleina. Hyperbilirubinemia esseva presente in 43%. Le tests de turbiditate a thymol e de flocculation a cephalina esseva anormal in 15, respectivemente in 9%. Le nivello del albumina seral esseva deprimite in 54% del patientes, durante que hyperglobulinemia esseva presente in 8.

Durante que le tensiones venose se monstrava in correlation positive con le grado del retention de bromsulfaleina ($r = +0{,}64$), illos esseva correlationate con le nivellos de transaminase solmente quando istos esseva anormal.

Le nivellos de transaminase glutamic-pyruvic esseva significativemente plus alte in patientes con disfallimento dextero-cardiac que in illes con disfallimento sinistro-cardiac. Isto suggere un connexion con le presentia de congestion hepatic. Le nivellos del altere enzymas monstrava le mesme grados de elevation in disfallimento cardiac dextere e sinistre, o illos esseva mesmo plus elevate in patientes con disfallimento cardiac predominantemente sinistre. Iste ultime observation suggere un relation con anormalitates metabolic plus generalisate que solmente le congestion hepatic de insufficientia cardiac.

PROGRESS
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UNDER THE CHARGE OF

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A FIELD STUDY OF DEATHS AND CAUSES OF DEATHS IN RURAL POPULATIONS OF THE PUNJAB, INDIA*

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DEATHS are a tool of the trade in public health work. A dependable knowledge of their number and nature is the main reliance in identifying the major health problems of a community. Deaths direct emphasis in programs for the treatment and control of community disease. They provide the background for preventive measures.

Similar information on morbidity helps materially, and is essential for those diseases with few deaths and a main influence on disability or residual defect: the common cold for example. Aside from the officially notifiable communicable diseases, that information is hard to come by and its collection is a costly process.

A mechanism for assembling deaths is established procedure almost everywhere in the world, although its efficiency varies. Death has the further

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advantage of being a readily identified and certain phenomenon; the count is easy. The means for determining its nature include the decisive test of necropsy[21]. Sickness, on the other hand, is not so sharply defined; the count is less exact, and complicated measures are often necessary to determine its nature. These are the main reasons that deaths have attained the significance they have.

The demographic problem that rests in increasing numbers of people and resultant population pressures finds deaths an outstanding consideration[12]. They are one of three elements that govern population numbers, the other two being births and migrations. On a short-term basis the death rate is the most sensitive variable: it can change rapidly in a short time due to improvements in environmental sanitation, provision of maternal and child welfare services, immunization, or the introduction of chemotherapy, antibiotics, and insecticides. By concentrating on a single disease, malaria, Ceylon[26] reduced its death rate within a decade from 20.6 in 1940 to 12.6 in 1950, thereby substituting a population problem for one of communicable disease.

For some countries and at particular times migration may rival the death rate, as in the case of Italy; it may be a local influence in the absence of any national significance. Almost never is the birth rate a significant factor in a short-term decline in population growth: the exception is Japan where birth rates within a decade fell from 33.5 in 1948 to 17.9 in 1958[15].

Deaths also reach more deeply into the life of a people than a simple relation to health and well-being. The money value of a man depends for one thing on how long he lives. Life expectancy also determines the age and sex distributions of a population, its virility and strength, attributes reflected in the social structure and the political progress of a nation. Cultural development has a hard struggle in the face of a high death rate.

Relative Reliability of World Data on Deaths. The recorded numbers of deaths in most countries of Western Europe and North America are highly reliable, the causes of death less so, particularly in rural areas where the quality and sufficiency of medical care is inferior to that of urban districts and confirmation by necropsy is infrequent. In technically underdeveloped countries the available data are likely to be incomplete and unreliable, often conflicting and frequently based on fragmentary coverage.

Between these extremes death rates vary greatly and unevenly; and to an appreciable extent within the two divisions themselves. Table 1 gives death rates and rates for infant mortality in selected countries of representative geographical regions. The data are chiefly from the most recent (1959) Demographic Year Book of the United Nations[32b]; some few are from the Annual Epidemiological and Vital Statistics of the World Health Organization[37b].

Not only do the rates vary in magnitude but they differ in reliability. The figures in the table in italics are unreliable or of unknown completeness. Numerous footnotes in the original publications qualify the data; they should be consulted in specific evaluation of the information given. The purpose here is to illustrate gross differences in frequencies and reliability. The two sources are in general agreement, with allowance for somewhat different divisions of populations and with deviations incident to the figures for so many countries being estimates. A measurement of the true state of affairs through intensive study of sampled

TABLE 1.—DEATHS PER 1000 POPULATION PER YEAR AND INFANT MORTALITY, DEATHS PER 1000 LIVE BIRTHS PER YEAR, FOR SELECTED COUNTRIES OF REPRESENTATIVE GEOGRAPHICAL REGIONS

	Year	*Death Rate*	*Infant Mortality Rate*
Northern Africa			
Tunisia	1958	20.0	173
Egypt	1956	*18.9*	*153.6*
Tropical and Southern Africa			
Belgian Congo (indigenous population)	1953	21.6	148
S. Rhodesia (indigenous population)	1954	14.4	122
North America			
U.S.A.	1959	9.4	26.4
Mexico	1959	11.6	73.7
Middle America			
Guatemala	1958	21.3	103.9
Barbados	1958	9.8	82.4
South America			
Brazil	1950	20.6	170
Chile	1958	12.1	126.8
Colombia	1958	*12.8*	*100*
Asia			
Burma	1955	35	*164.3*
Federation of Malaya	1958	11.0	79.6
Indonesia	1950-54	20	150
India	1958	19.4	*185*
Japan	1958	7.5	34.6
Thailand	1956	*9.8*	55.2
Europe			
United Kingdom	1958	11.7	23.2
Sweden	1958	9.6	15.8
Poland	1958	8.4	72.8
Italy	1959	9.3	44.9
Spain	1958	8.7	47.4
France	1959	11.2	25.2
Fed. Republic of Germany	1959	10.8	34.3
Oceania			
Australia	1958	8.5	20.5
New Zealand	1958	8.9	23.4
Gilbert and Ellice Islands	1956	25.0	124.6
U.S.S.R.	1958	*7.2*	*40.6*

Source: Primarily Demographic Year Book of the United Nations[32b]; in some instances Annual Epidemiological and Vital Statistics of the World Health Organization[37b]. Italics indicate that data are unreliable or of unknown completeness.

populations clearly has value in many pre-industrial countries.

An opportunity to acquire that sort of information in partial degree and for one country presented itself incident to a long-term prospective investigation of population dynamics[11] in a rural area of the Punjab, north India. These studies began in 1953 and ended in June, 1960. Because changes in the size of populations are the result of altered relationships between births, deaths, and permanent migrations into or out of an area, deaths had special attention.

A few items of information added to those required of the primary investigation also permitted base line facts about health affairs of fundamental value as a guide to preventive measures and useful in evaluating existing public health practices. The study resolved into the following objectives.

The first was to determine accurate death rates through direct field observation, and to compare them with the official rates reported by health agencies from routinely collected statistical records. The purpose was to determine whether or not significant differences existed; and if such were the case to develop a means for better estimates. A second consideration was of trends in death rates over time, to facilitate projections of future population movements.

A third interest centered in causes of death, determined through individual case study. The nature of diseases commonly ending in death bears importantly on probable death rates of the future and so ties in with the demographic problem. An equally important consideration is the usefulness of such information in public health practice. Causes of death as determined were again compared with officially recorded causes, and an investigation made of medical care during terminal illnesses in relation to the usual seriousness of the disease and its amenability to treatment.

Differences between individual villages of the study area were investigated in respect to crude death rates. Such demographic features of deaths as age and sex, and those related to selected social and environmental factors had attention in an effort to determine the long-term changes in numbers and character of deaths. Short-term variations also were studied, namely the nature of fatal illnesses at different seasons of the year.

Death Rates in India. The local study area at Khanna is now related to Ludhiana District, of which it is a part, and the district in turn to the Punjab State and the Union of India. Currently reported death rates and trends in rates over a period of years are given for the three administrative levels. Brief comparison is made with other countries, selected in the main to illustrate high and low rates.

Crude Death Rates. Ludhiana District is located in the center and to the north within the Punjab state; it has a population of 808,105 by 1951 census and an area of 1279 square miles. The general death rate, as reported by the Directorate of Health Services of the Punjab, was 14.6 per 1000 population in 1959.

The Punjab state itself is in Northwest India. The population by 1951 census was 11,659,013 within an area of 37,378 square miles. A rearrangement of state borders has added population and area so that the mid-year 1958 estimate is of 17,849,300 persons. Reported death rates were 15.0 in 1958 and 12.2 in 1959. Crude death rates are subject to variations by geographical, social and other environmental characteristics which conceivably could account for differences between the Punjab as a whole and the district of

Ludhiana. As a matter of fact, 5-year averages of death rates for the one are within the standard deviation of the other.

Information on national rates varies within wide limits. The World Health Organization[37b] quotes a rate for India of 9.1 for 1956 based on official returns. The Statistical Abstract of India[14d] gices a rate of 11.7 for 1955. The 1959 edition of the Demographic Year Book[32b] quotes 11.9 for the registration area in 1958, with a note that the figure was not computed by the Statistical office of the United Nations. This edition also gives an estimated rate of 19.4 in 1958 for rural India, taking advantage of all available information, particularly the 14th round of the National Sample Survey; the population of India is 82.7% rural. Rates for all India[4] estimated by the reverse survival method from the 1941-1951 census returns[14b] were 27.4. Chandrasekhar[3] cites a recorded rate for 1941 to 1951 of 20 and an estimated rate of 27 for the same period, in general agreement with Kingsley Davis[8].

The National Sample Surveys[14a] forcefully altered ideas about crude death rates for India as a whole. Under non-epidemic conditions, they are rather assuredly greater than usually quoted. The situation in the Punjab is less definitely known but probably the generalization holds.

Infant Mortality. The Directorate of Health Services of the Punjab gives the infant mortality rate for Ludhiana District in 1959 as 109 per 1000 live births and for the Punjab a rate of 100. These rates appear low in comparison with national rates, although again available data for all India show considerable differences.

The Demographic Year Book 1959[32b] quotes a recorded rate for the registration area of India of 96.1 in 1958, and a rate of 185 for all India for the same year based on demographic analysis of the 1941-1951 census returns. The World Health Organization[37b] gives a rate of 89.9 for 1956, the Statistical Abstract of India[14c] 100 for 1955, and Chandrasekhar[3] a recorded figure of 119 for 1953. Infant mortality rates for India are high, whatever the value taken as authentic. They are by no means extreme; apparently they are about midway between estimated frequencies in primitive peasant societies and the better rates among countries of Europe and North America[32a,34].

Van der Hoeven[17], for instance, reports a rate of 230 per 1000 live births on the north coast of Netherlands New Guinea. According to Jelliffe[19] infant mortality in most of Africa is generally in the region of 300 to 500. The infant mortality among children of employees of an oil company in Burma[9] was 163.4. Most countries of Asia and South America occupy a middle position. In an analysis in Ecuador, Garcia Arroyo[10] found a rate of 108.1 for 1956.

Present conditions in India do not differ greatly from the United States in 1900, when Bogue[1] reports that of a cohort of 1000 children 834 survived to enter kindergarten. The recent study of Wallace *et al.*[35] shows how the situation has changed. An analysis of death certificates of resident infants and preschool children in Minneapolis who died between 1952 and 1956 gave an infant mortality of 22.2. The State of Tennessee[2] quotes infant and neonatal mortality rates as 30.3 and 21.2 respectively in 1959. Progress has been general[18] to give the United States a position among countries with favorable rates for deaths of infants.

Neonatal Death Rates. Current official rates for neonatal deaths in the Punjab and Ludhiana are respectively 48 and 60 per 1000 live births per year. The Poonamallee Survey[29] of 1951 in Madras, South India, gave a rate of

45.7. The recorded neonatal death rates for Ludhiana District and the Punjab, taken in relation to reported infant mortality, suggest that one or the other is too low, and perhaps both.

Knowledge of the neonatal death rate has practical significance. The impact of modern public health measures is evidenced almost universally in the changes that come about as public health improves are well illustrated by the studies of Craig in Aberdeen[6]. In 1900, infant mortality was 149 and deaths in the second year of life 169 per 1000 population per year. In 1949 the corresponding rates were 30 and 11. A similar change has occurred in the United States. Figure 1 shows proportionate mortality according to age in states of the registration area of 1905 and for the same states in 1958. Data for India as of 1957 resemble those of earlier United States.

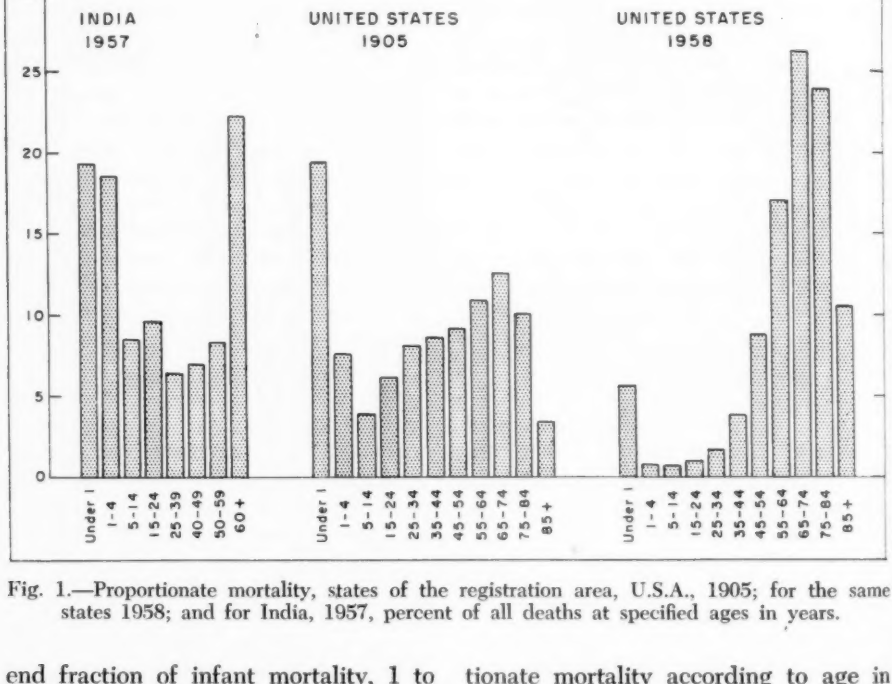

Fig. 1.—Proportionate mortality, states of the registration area, U.S.A., 1905; for the same states 1958; and for India, 1957, percent of all deaths at specified ages in years.

end fraction of infant mortality, 1 to 11 months, with much less progress in the neonatal period of the first 4 weeks. It is important to separate the two. An improved infant mortality is indicative of results; the postneonatal death rate is the more precise measurement.

Age-specific Death Rates in Early Childhood. Abnormally high death rates in early childhood, aside from infant mortality, are found in countries with limited health facilities. The

Excessive death rates in the early years of childhood are best illustrated in primitive agrarian societies. As usual, infant mortality is the major consideration. Too often attention centers on that factor, to the neglect of the appreciable mortality associated with the

years immediately following. Jonkers[20] in a study in Liberia found that about 45% of children born under native village conditions died before they could walk and only 40% lived to reach the age of 12 years. Infant mortality was estimated at between 400 and 450 per 1000 live births. In the Kankara District of Northern Nigeria[16a] deaths among children 3 years or under accounted for 45% of total deaths, results subsequently duplicated in Sierra Leone[16b]. An epidemiological survey of the Gola tribe in Liberia[25] brought out that only 20% of pregnancies resulted in a child who reached the age of 6 years. Macdonald[23] states that in parts of Africa the proportion of children surviving to adult life is 50% compared with 95% in Britain and the United States; the cumulative mortality up to 15 years of age was about 10 times higher in Africa than in the two Western countries mentioned. The mortality among pre-school children in Guatemala[30] accounts for between 70% and 90% of total deaths in the Coastal Lowlands and 10% to 30% in the Central Highlands.

Efforts to reduce the general death rate in pre-industrial countries are most effective when they center on the early childhood ages. An index of improved health conditions and especially of nutrition is proposed, based on the ratio between deaths in the second year of life and that in the post-neonatal period adjusted to an annual basis. Unfortunately, data on deaths by individual years to age 5 are not often available even in countries with good vital statistics; the usual practice is to group ages 1 to 4 years.

Trends in Death Rates. The Khanna study produced data on the size, rate and direction of change in death rates for a relatively short period of 4 years. It is pertinent to know how the results correlate with preceding experience, and in turn how behavior in the local area compares with that in larger units of the Indian population. Figure 2 shows trends in death rates in Punjab state for the past half century. Those for Ludhiana District and for all India are in such close agreement that illustration is not profitable. Between 1901 and 1959, death rates recorded in Ludhiana District declined from 69.2 to 14.1, in the Punjab from 45.2 to 12.2, in all India, 1901 to 1955, from 34 to 11.7. As brought out in the discussion of crude death rates, the absolute figures are probably less than fact. For the purposes of determining trends they serve acceptably because over the years the margin of error presumably was within much the same limits.

The conclusion is evident that in India and these subdivisions an improved public health has brought a rather remarkable decline in death rates, especially during the past two decades. As the general health situation improves, the more difficult problems are left. It becomes increasingly necessary to identify them, through knowledge of the particular diseases responsible for deaths.

Causes of Death. The frequency and place of death define its extent; an understanding of its nature depends on knowledge of the particular diseases involved, the causes of death. In many countries one cannot expect to know much about cause with the sketchy and fanciful information about the actual frequency of the event itself. In a study of African children in Southern Rhodesia[7] the chief cause of death was given as prematurity; other causes listed such things as swellings and sores, fever, and cough. Yasse[38] reported the principal causes of death in children of the Congo, in order of importance, as respiratory infections, debility, fevers and chronic infections, and diseases of the diges-

tive system. This is not particularly helpful in planning public health programs.

India has well ordered information about causes of death in urban centers, largely derived from the teaching hospitals of medical schools, but biased by the selected nature of the population of patients. Some metropolitan areas, notably Bombay[13], have made progress in collecting data for the community as a whole. However, the India of today is primarily an agrarian society, with village populations the major element.

The information on causes of death for rural communities comes mainly from surveys made in demonstration health centers which scarcely can be taken as typical. The aim of the Poonamallee Survey[29] for example was to assess the true infant mortality and the causes of such mortality in a rural area of Madras state. Cohort methods were employed. For the period under inquiry, August, 1951, to August, 1952, infant mortality rates ranged between 137.6 and 347.8 per 1000 live births, to give a rate for the cohort in the first year of life of 140.7. The leading cause of death was intestinal intoxication, 22.6%; asphyxia was next, 14%, and prematurity, 12.4%, followed by bronchitis, pyogenic infections and nutritional maladjustments.

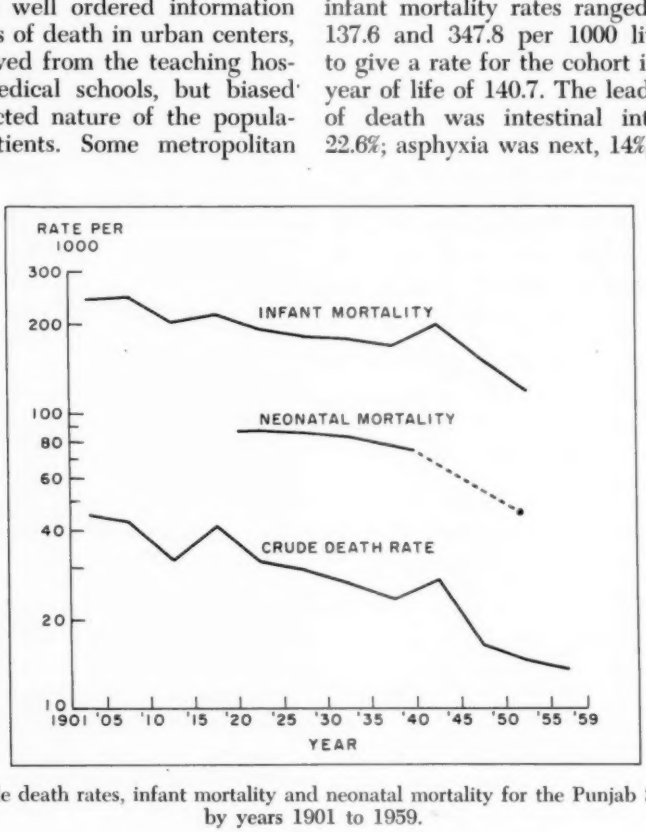

Fig. 2.—Crude death rates, infant mortality and neonatal mortality for the Punjab State, India, by years 1901 to 1959.

The Punjab and Ludhiana District list only 8 simple categories of reportable causes of death, Table 6. This classification is all that is possible because individual deaths are characterized in most instances only by a symptomatic diagnosis in terms such as fever or cough. The reporting of deaths in the usual village community is a police function rather than by medical certification, for the practical reason of

relatively few qualified physicians in village practice.

Disease in rural areas tends to have its individual characteristics. Facts about prevalence and killing capacity of the commoner conditions, determined with care as they apply to city dwellers, are not an infallible guide to what happens in villages. Diseases of little importance in cities, or in countries with good health facilities, are often outstandingly important in the rural areas of pre-industrial nations. For example, tetanus is common in Jamaica children[27] and ranks tenth among leading causes of death for children of Mexico[22]. Senecal[28] reports 241 cases of tetanus in a hospital in Dakar, French West Africa, 103 of which were tetanus neonatorum. In other rural situations, because of environmental conditions or inadequacies in medical care, a disease such as malaria[5] becomes established as an endemic process out of all proportion to its usual activity.

Some other maladies are predominantly rural in their distributions because of occupational influences, as with brucellosis. New diseases often appear for the first time in rural situations because of the existence there of the environmental conditions that make them possible; Kyasanur forest disease[36] for example.

The usefulness of an orderly determination of numbers and kinds of deaths in village communities is evident. The field studies now to be described were directed to that end.

The Study Area. Eleven villages near Khanna in Ludhiana District, the Punjab State, India, having a population of about 12,000, were observed for 4 years. When observations started in 1956, the largest village had 1867 residents and 278 persons lived in the smallest community. The usual village has from 800 to 1000 inhabitants.

A characteristic feature of Indian rural life is that farmers of a region all live within a village. From there, they go out to work the land. There are no independent farm residences as in many parts of the world, notably the United States. Consequently the population of the villages is collectively the rural population.

Official Reporting of Deaths. The "chowkidar" or village watchman has the responsibility to collect reports of deaths and transmit them to higher authority, in this instance through the police to the Health Services of the Punjab. The chowkidar is the lowest official of government, with an imposing list of duties that range from the reporting of crimes to the collection of vital statistics. A village may have one or more chowkidars according to its size and economic status, or two small villages may share a chowkidar. Five of the 11 villages of the study area had 2 chowkidars and the remainder one each; in all, 16.

The chowkidar is one of the people of the village. His obligation about births and deaths is to record all such events occurring within his particular jurisdiction, including causes of death and the results of deliveries. Once each two weeks he takes his birth and death registers, books of simple standard forms, to the police station of the area. Copies of his records are made there, sent to the District Civil Surgeon, and eventually transmitted to the Directorate of Health Services at the State capital by the District Medical Officer of Health.

While legally responsible for reporting births and deaths, how the chowkidar does it is more or less his own business. He has no organized course of training in recording numbers and causes of death, nor does he know much of the value and utility of accurate vital statistics. The office is ap-

pointive and the qualifications relate more to his other duties. By custom, the appointment is often hereditary which serves to an extent in preparation for the work.

With few exceptions the chowkidar is illiterate. Consequently he takes his books and his information to some member of the community who can read and write and that person makes the record: a shopkeeper, the school teacher or some other. The lambardar or local tax collector then attests the record. Since the report to the police is on a fixed day every second week, the usual practice is to complete the record the day preceding. The possibility therefore exists that some vital events are forgotten or that dates may be entered incorrectly, although such errors are fewer than might be expected.

The sources of his information are irregular and unorganized. Informants usually lack technical competence as to causes of death and especially such specific information as to whether a delivery resulted in a live birth and the child shortly died, or was a stillbirth. Knowledge that a death has occurred usually comes in the course of his broader duties, his trips through the village, sometimes from relatives or friends of the deceased, from direct observation of funerals, or from the general gossip that permeates these little villages. There are no qualified physicians in any of the villages of the study area, and the majority of deaths are unattended by a physician. Consequently the leading cause of death ordinarily is expressed by a prominent symptom as interpreted by the family or other attendant.

It is to be emphasized that the system of reporting is on an area basis; the record is that of all deaths occurring within the geographic bounds of the village and its surrounding fields. If a resident of the village dies outside the village area the death is not charged against the village population. If a stranger dies in the village, that becomes a village death. There is no means to determine the number of deaths attributable to the actual population of the community.

Survey Detection of Deaths. Case study of all deaths within the study area was an integral part of the investigation of population dynamics. This was done independently of the official procedure, first because of the need for assured accuracy, and secondly to permit comparison of numbers and rates.

The main reliance in case finding was through the routine activities of the population dynamics study staff. The resident woman village worker visited once a month all families where the wife was of child bearing age, 15 to 45 years. The male worker interviewed husbands wherever he found them, in the fields, at the irrigation wells, at home or in the informal gatherings of an evening so much a part of village life. Through long residence the village workers became a part of the community. This relationship greatly favored accurate information, especially as mutual confidence and understanding were established.

A staff physician visited each village once a week. No organized medical care was provided through a clinic or otherwise; the interests were in the population study. On request, medical services were given. In this way, a goodly proportion of serious illnesses was seen, thus identifying them and giving opportunity to determine their nature.

Deaths occasionally came to light through observed activities at the burning ghats in the course of the customary cremation.

As informative a source as any was

the round of village gossip coming to the attention of resident staff members. In the general absence of newspapers, telephone, or other means of common communication, this is decidedly active. It took form as an organized effort through appointment of so-called informants, men and women of the village who voluntarily assumed responsibility for reporting vital events for a prescribed group of 25 to 30 neighborhood families. They reported once weekly to the village worker, and additionally as events occurred. The number of informants varied from 4 to 8, according to the size of the village. This system proved most effective.

The second datum essential to reliable death rates is an accurate count of the population at risk. Demographic information on all residents of the village was maintained through a continuing family roster, the conventional family folder of field epidemiology. Additions and deletions by birth, death or migration were brought up to date as a part of the monthly family visits. This information was checked once a year by actual census of each village.

Assigned Cause of Death. Every death in this study was investigated by a staff physician. The evidence as to probable cause was from several sources; they were of varying reliability. Some patients had consulted a staff physician during the last illness. Attendants at the time of death and sometimes in preceding disabilities were interviewed in most instances. Sometimes this was a qualified physician, more commonly a practitioner of indigenous medicine, a midwife, or many times one of the village people with a local reputation in the healing art. Not uncommonly no more than a friend or relative gave aid. As is always the case, some patients had no medical attention of any sort, as in unexpected death by accident or a person found dead. Without exception, relatives were interviewed after the death as to the nature and course of early and late manifestations.

Where patients died in hospital or had been in hospital shortly before death, advantage was taken of records of clinical, roentgenographic and laboratory examinations and the attending physician was consulted whenever practicable. No diagnosis in this series was confirmed by necropsy; examination after death is not common in general medical practice in India. Other than for patients in hospital, the aid to be had from laboratory procedures was minimal; facilities are close to nonexistent in village practice. Reliance was mainly on clinical examination, and the history of illness.

After investigation in the field, each death was reviewed at a clinical conference of 2 to 4 physicians and the cause of death established on the basis of all clinical, laboratory and epidemiological findings. The decisions taken were recognized as of varying reliability, sometimes because of the nature of the disease and sometimes because of incomplete information.

The World Health Organization[37a] approaches this problem by recommending separate classification of deaths for areas where medical certification is deemed adequately complete and those where it is not. This procedure was not applicable because no deaths were medically certified. The principle still was followed in that deaths were divided into two groups, those where the patient had been seen before death by a qualified physician and a diagnosis was available through his clinical records or through interview, and secondly those where no doctor of medicine had attended.

After assigning tentative causes of death, cases were further subdivided according to reliability of diagnosis.

Some were judged relatively certain, others of doubtful validity, and for some the cause was considered unknown. Doubtful cases usually had strong suggestion of a leading cause of death but another disease of similar nature could not be excluded. Deaths were recorded as of unknown cause when data were poor, incomplete, or in a few instances wholly lacking.

Coding of causes of death was according to the International Classification of Deaths and Disease[37a]. The several categories were then distributed according to the "B" or short list of that publication. The ten leading causes of death were determined separately for those deaths under medical care and for the series as a whole. The generous and valuable aid of Dr. Enid Charles of the World Health Organization, Regional Advisor in Health Statistics for Southeast Asia, New Delhi, contributed materially to the authority of decisions taken in assigning causes of death.

Allocation of Deaths and the Basis for Rates. The broader investigation of population dynamics required knowledge of the number of deaths occurring in the population directly at risk, the normal residents of the villages. A second purpose was to compare observed death rates with those obtained through routine channels, the objective being to derive a factor useful in estimating the actual death rates of other communities within the same general region.

For these reasons survey deaths were allocated by place of occurrence and also by place of residence of the deceased. This required collection of deaths of residents dying outside the village, as well as all village deaths. Two groups were thus distinguished, area deaths and residential deaths.

Area deaths include all fatalities occurring within the geographical confines of the village, whether of residents or of non-residents. The official death rates are calculated on this basis, populations being from the 1951 decennial census, statistically corrected for the intervening years. The inherent assumption is that deaths of non-residents within the village are balanced by deaths of residents in other localities. Collected in this way, survey and officially reported results are comparable either by actual numbers of deaths or by death rates, providing the same population serves for both.

Residential deaths include all area deaths, less those of non-residents and plus those of residents dying in other localities. Arbitrary definition of residents and non-residents is necessary. The strict village-wise exogamy practiced in the Punjab results in more than usual movement of population. Wives of the village customarily return to their home village and their parents for the birth of the first baby and sometimes for subsequent deliveries, in such instances taking young children with them. Likewise, women born in the village but living in another community return temporarily to their native village. The distances are many times great and commonly outside the study area.

A resident death thus was considered to be that of any person who had lived in the village at least six months before death or once having been a resident, had not stayed out of the village for six months or more before death. This interpretation took care of the short-term migration of pregnant wives into and out of a village.

Births were allocated in the same fashion as deaths, with area births or residential births used appropriately in calculation of neonatal and infant mortality rates. The special studies began in April, 1956. To permit adequate comparison between reported and ob-

served deaths, many of the analyses to follow are based on the three calendar years of 1957, 1958 and 1959.

Observed Crude Death Rates. Crude death rates for the eleven villages are presented in Table 2. The population used in calculation of rates was that derived by annual census, adjusted to mid-year, and all deaths were verified by case study, except that estimates were necessary for nine villages during the first 3 months of 1956 because full field operations did not come into force until April. These particular estimates were by family interview and checked by the village register of births and deaths as kept by the village watchman.

The death rates in Table 2 are presented in two ways, as rates for residents of the village (residential death rate, col. 4) and as an area death rate (col. 6). The two procedures have been defined. The residential death rates represent the true mortality of the village populations at risk. The fallacies inherent in area death rates are long recognized; for instance, to give favorable rates for suburbs of the United States and high rates for adjacent cities where people go for hospital care of serious illnesses.

In these village populations and under existing conditions, the two sets of rates are almost identical. Minor variations are evident for a given year but the end result over the four-year period was in close agreement, 17.0 for residential deaths and 16.8 on an area basis. It is evident that deaths among non-residents effectively cancel out deaths of village residents in another locality. The important and individual factor in these populations, the temporary exchange of pregnant wives between communities, is apparently an even matter.

The area death rate, much simpler to obtain and the existing practice, gave as workable an indication of the frequency of deaths as the theoretically more desirable allocation by residence. How well this works out in practice in the routine registration of deaths is another matter, to be considered subsequently.

Considerable variation in crude death rates from one year to another would be expected, where communicable disease is as prevalent as it is in this general part of the world. No outstanding epidemics occurred during the study period and total annual death rates were at much the same level. The small populations and the presence of local outbreaks of the common communicable diseases resulted in appreciable variations in annual death rates for individual villages, Table 3; as, for example, a rate of 12.8 for Manupur

TABLE 2.—DEATHS AND DEATH RATES IN ELEVEN VILLAGES OF LUDHIANA DISTRICT, PUNJAB STATE, INDIA, 1956-1959, BY PLACE OF RESIDENCE (RESIDENTIAL DEATHS) AND BY PLACE OF OCCURRENCE (AREA DEATHS)

Year	*Mid-Year Population by Survey*	*Deaths among Residents*	*Residential Death Rates*	*Area Deaths within the Villages*	*Area Death Rates*
1956	12,022	206	17.1	222	18.5
1957	11,998	195	16.3	196	16.3
1958	12,111	217	17.9	200	16.5
1959	12,199	203	16.6	194	15.9
All years	48,330	821	17.0	812	16.8

village in 1957 and 22.5 in 1958. The villages of Bhari and Dhilwan had rates higher than average, and others had low rates: the villages of Gowadhi and Rajewal, the latter particularly so in comparison with its near neighbor, Kulewal. The smallest village, Dangorey, population 278, had the best

from deaths among the infants actually involved, is possible by cohort study which was done as another part of these investigations, but the findings are not presently available.

The rates for neonatal deaths, Table 4, are high, 73.9 per 1000 live births per year; and yet they account for

TABLE 3.—RESIDENTIAL DEATH RATES IN THE LUDHIANA STUDY AREA 1956-1959, BY INDIVIDUAL VILLAGES

Village	*Village Population 1956*	*Death Rates per 1000 Population per Year*			
		1956	*1957*	*1958*	*1959*
Manupur	1478	20.3	12.8	22.5	14.8
Kotla	997	21.1	12.6	21.4	18.5
Bhari	1434	16.7	27.0	15.3	23.1
Manki	1737	19.0	15.9	20.5	18.2
Rajewal	993	14.1	17.3	17.0	13.1
Kulewal	714	21.0	12.6	18.1	23.6
Dhilwan	507	21.7	16.7	20.0	16.0
Sarinh	1370	10.9	18.6	9.9	14.7
Gowadhi	1867	16.6	13.7	17.1	15.8
Jarkhar	647	17.0	15.4	22.2	7.3
Dangorey	278	3.6	10.5	13.4	13.3
All villages	12,022	17.1	16.3	17.9	16.6

TABLE 4.—NEONATAL AND INFANT DEATHS AND DEATH RATES (RESIDENTIAL) IN ELEVEN VILLAGES OF LUDHIANA DISTRICT, PUNJAB STATE, INDIA, 1956-1959

Year	*Live Births*	*No. Deaths 0-27 Days*	*Neonatal Mortality*	*No. Deaths 0-11 Months*	*Infant Mortality*
1956	466	35	75.1	76	163.1
1957	457	34	74.4	65	142.2
1958	465	35	75.3	78	167.7
1959	480	34	70.8	83	172.9
All years	1868	138	73.9	302	161.7

record, and regularly so, despite the potential effect of a few extra deaths among such limited numbers.

Observed Neonatal Mortality. Neonatal death rates were calculated by the standard procedure of annual deaths per 1000 live births of that year. The minor fault in that method is recognized[33], for deaths of a particular period are charged against a population of another. The more exact rate,

only 45.7% of infant mortality. For purposes of comparison, the neonatal death rate in the United States for the years 1954 to 1958 was 19.1 per 1000 live births per year; that was 72.3% of total infant mortality.

Observed Infant Mortality. In view of the neonatal death rates just cited, infant mortality in these Punjab villages would be expected to be high and in excess of usually quoted rates, Fig. 2.

The average rate for the 4-year period was 161.7 per 1000 live births per year, Table 4.

The two rates, neonatal and infant mortality, do not parallel each other in individual years; the lowest neonatal death rate of the four years was in 1959 and that year had the highest infant mortality; the year 1957 had a relatively high neonatal rate and the lowest infant mortality. In general, the behavior of both sets of rates is of reasonable regularity from year to year.

Infant mortality is clearly marked as the main factor in the relatively high crude death rates of these communities. The average age-specific death rate per 1000 population per year for persons aged 1 year or more was 11.1. This compares favorably with the rate for the same age group in the United States during equivalent years, namely 8.9 per annum.

A Comparison of Officially Recorded and Survey Death Rates. During the four years 1956 to 1959 the survey turned up 812 area deaths, and 818 were reported through routine channels. Death rates are almost identical, 16.8 for survey deaths and 16.9 for deaths routinely recorded, when the same population is used, that of the survey. If the two rates are derived each from its own elements of deaths and populations, the 1951 census figures being adjusted for natural population increase, the rate from official sources is appreciably higher, 19.9 per 1000 population per year, against 16.8 for survey deaths and population.

An adequate and simpler comparison of the two procedures is through actual deaths. For this purpose, deaths during 1957, 1958 and 1959 are used, for reasons already stated. During this period the chowkidars registered 603 deaths as occurring within the village areas, while the survey total was 590. Seemingly either source is fairly indicative of the true state of affairs. That is not the case. Errors in the routine data are numerous, various and diffuse. While they come close to cancelling out as far as total numbers are concerned, they leave a false idea of important demographic features.

Although the routine count yielded 13 more deaths than did the survey, 44 deaths identified by survey were missed by the chowkidars; 546 were in both series. The error is 7.5%. The omissions included only 12 males; there were 32 females. Most of the omitted deaths were of persons aged less than one year, 31 in all, while 13 were past that age. Of those under 1 year, 7 were males and 24 were females. They are further divided into 15 in the neonatal period of 4 weeks, and 16 in the succeeding 11 months. Still another peculiarity was that while deaths by sex were much the same in the neonatal period, 6 males and 9 females, the distribution within the succeeding 11 months was a single male death omitted and 15 female. After the first year of life there was fair agreement, 5 males and 8 females.

An additional 22 deaths in the chowkidar series were registered as occurring in the villages, but in fact the deaths were outside the community. The remains of 14 one-time residents were returned to the family village for cremation or burial. One person, a resident by stated definition, died outside the village but was entered among deaths within the area. Presumably all of these deaths had been registered previously at the place where they occurred. Five deaths were among a group of nomads camped near one of the villages, and not a part of the population. The circumstances for the remaining 2 could not be determined; the names were not on the family rosters maintained for all persons resident in the village, nor could they be identified

by inquiry or other means. The totals for the study period included a further 4 deaths of residents which occurred in other years. In all, the series included 26 excess deaths not properly chargeable to the community on a time and place basis.

Six abortions and 25 stillbirths, among the 153 abortions and 52 stillbirths identified in these studies, were recorded as deaths of living infants. The sex distribution of those so reported was unusual. Males ordinarily predominate but the division of 24 males and 7 females was clearly unusual. The difficulty in determining sex in abortions may account for a part of the disparity, but cultural inclination such as "had he lived he would have been a fine, big boy," seemed the more important.

With these corrections the two series are in agreement, 590 deaths during the 3-year period. The crude death rates observed in the study area are now compared with those of Ludhiana District, of the Punjab state, and of the Union of India.

The rural areas of Ludhiana District are sufficiently homogeneous that the study area may be considered representative of the region. The presence of the survey workers in the villages and knowledge by the village watchmen that data on births and deaths were being collected undoubtedly stimulated effort on their part. The results from these villages scarcely can be taken as usual or ordinary. The better comparison is with Ludhiana District.

The nicety of excluding the study area seems unnecessary, because a difference in death rates for its 12,000 population of any ordinary proportions scarcely would be reflected in the larger population of 808,105. One possible comparison is of the crude area death rate of 16.8 determined by survey with the official rate for the district as a whole. In much the same years, the data were for 1953 to 1957, the death rate for the district was 14.9. Since the population of the study area as shown by survey is appreciably greater than by the 1951 national census, adjusted for natural increase, the better comparison is by computing the death rate for the study area using the official census. The rate is then 19.4 per thousand population per year, a difference of 4.5 between the two rates. On these bases, deaths are underreported at least 13% and perhaps as much as 30%.

It is reasonable to assume that deaths are better reported in metropolitan centers than in the rural regions. Excluding Ludhiana City, the population of Ludhiana District is 624,452 in 1959 and its crude death rate 16.0. The higher level would perhaps be more typical of rural areas. The correction would then be 21%.

Punjab State for the years 1953 to 1957 had an average annual death rate of 14.1 per 1000 population per year, in close agreement with Ludhiana District. With less assurance, the stated corrections could be extended to other rural populations of the state.

The Demographic Year Book[32b] cites the average crude death rate for India as 13.4 for the years 1951 to 1955. Since the population of India is predominantly rural, it is reasonable to believe from these field studies that underreporting of deaths is more or less universal. The nature and extent presumably vary from one locality to another because of differences in customs, culture and the physical environment. The projection of these observations to the larger sphere is unwarranted. The need is indicated for other surveys of similar nature in representative parts of the country.

The particular shortcomings in re-

cording of deaths, as brought out earlier, were in the first year of life. A discordance between infant mortality and neonatal deaths also was noted; the infant mortality appeared low with the crude death rate what it is. The evidence supports an underreporting of infant deaths.

The recorded infant mortality rate for Ludhiana District for the years 1952 to 1957, approximately those of the survey, was 112; for the survey series it is 161.7 for 1956 to 1959. The corrective factor in respect to infant mortality would be 44% on the basis of survey rates, this proportion to be added to the number determined in the course of routine recording.

Infant mortality rates for the Punjab State for 1952 to 1957 were 109, in essential agreement with those for the District, with the corrective factor 48%. As stated in respect to crude death rates, these observations in the Punjab cannot be applied with any reasonableness to India as a country. However, the generalization about underreporting of infant deaths appears tenable.

The differences in regard to neonatal mortality are of much the same order as for general infant deaths. The survey results gave a rate of 73.9 per 1000 live births per year. The frequency for Ludhiana District for the same years stated above was 56 and for the State it was 59. The factor applicable to these rates is about 32% for the District and 25% for the State.

Causes of Death by Case Study. Because of the system of reporting, causes of death as routinely notified are highly inaccurate, indefinite, and expressed only in general terms. The recorded causes of death for 603 fatalities registered by chowkidars during 1957 to 1959 are presented in Table 5.

The leading cause of death is Cough and Fever, followed closely by Fever and Weakness; together they account for two-thirds of deaths. Other causes are scattered within a wide range. The fourth leading cause is *"athrah,"* or Eighth Disease, a term translated with difficulty and variously interpreted: by

TABLE 5. — OFFICIALLY REPORTED CAUSES OF DEATH IN ELEVEN VILLAGES OF LUDHIANA DISTRICT, PUNJAB, INDIA, JANUARY, 1957, TO DECEMBER, 1959

Cause	*No. of Deaths*
Cough and fever	208
Fever and weakness	204
Pneumonia	17
Athrah and fever	15
Asthma	14
Loss of blood	14
Accidents	12
Marasmus, fever, cough	12
Fever, dysentery	10
Cough	8
Old age and weakness	7
Did not suck breast	6
Stroke	5
Measles	5
Tuberculosis	4
Fever, cough, old age	4
Fever, cough, asthma	3
Fever, diarrhea	2
Boil and fever	2
Flatulence, distended abdomen	2
Pain in abdomen	2
Malaria	2
Died after delivery	2
Lock jaw	2
Fever and pain in abdomen	1
Fever, cough, *athrah*	1
Palpitation of heart	1
Murder	1
Operation, abdominal	1
Epilepsy	1
Heart failure	1
Bleeding per vagina	1
Cholera	1
Asthma and swelling	1
Fever and difficult respiration	1
Smallpox	1
Urine difficulty	1
Postpartum fever	1
Tumor	1
Carbuncle	1
Cause unknown	25
	603

some villagers as death following birth in the eighth month of pregnancy (prematurity) and more commonly as a disease invariably fatal with death on the 8th day of life and associated with convulsions. In the Western Isles of Scotland in the 19th century tetanus was a common disease, known colloquially as Eight Day Fever: *athrah* fits well.

Cholera, plague and smallpox are well known in the villages, and are reported as such. Within living memory the area has been the site of great epidemics of plague. Several of our villages still maintain shrines to the goddess of smallpox, and cholera has visited the region repeatedly. Despite its stirring history, the Punjab has been singularly free of these great internationally quarantinable diseases for many years; a single case of smallpox was the sole occurrence in this experience. Among the few other medical conditions recognized and sometimes reported by specific name are pneumonia, malaria, measles and tetanus, all communicable diseases.

The patient may have been seen by a physician of a neighboring town or city; it remains for the chowkidar to register the cause of death. There are no resident physicians in any of the study villages. The list just given evidently contributes little to the potential gains from a knowledge of causes. The challenge to survey methods is all the more inviting.

The reports from the villages, for the most part symptomatic diagnoses, are condensed by district and state authorities to a simple classification, uniform at both jurisdictional levels. Table 6 illustrates the findings for Ludhiana District in 1959. The main category is Fevers, which offhand might be interpreted as heavily weighted with malaria; although once true, that infection is now close to a curiosity in the Punjab. The ordinary communicable diseases are the main feature, along with a goodly portion of secondary and terminal infections.

TABLE 6.—DEATHS AND DEATH RATES, LUDHIANA DISTRICT, PUNJAB, INDIA, 1959, BY CAUSE OF DEATH

Cause of Death	*No. Deaths*	*Deaths per 1000 Population*
Cholera	0	—
Smallpox	4	0.004
Plague	0	—
Fever	6299	7.74
Diarrhea and dysentery	230	0.28
Respiratory diseases	2282	2.81
Injuries	238	0.29
Other causes	2856	3.51
Total	11,909	14.64

Source: Directorate General of Health Services, Punjab, India.

The deaths of Table 5 are the source material for the case studies of this investigation, plus some residential deaths outside the villages. Fetal deaths are excluded; all others are included. The validity of the basic data bears strongly on the results to be presented. This was appraised with frank objectivity, collectively by the group of physicians associated in the work, by methods already given. The distribution of cases according to reliability of diagnosis is in Table 7.

A cause of death was established with relative certainty for about 65% of cases, 29% were doubtful and 6% were classed as unknown. Cases attended by a physician had a higher proportion of certain diagnoses, 77%, than those without medical care. There were no instances of cause unknown in that group, for the principal reason that persons found dead or having no attendance other than by relatives were automatically excluded. Diagnoses were deemed certain for cases without quali-

TABLE 7.—RESIDENTIAL DEATHS IN ELEVEN VILLAGES OF LUDHIANA DISTRICT, PUNJAB, INDIA, FROM JANUARY, 1957, TO DECEMBER, 1959, BY PRESENCE OR ABSENCE OF MEDICAL CARE BY A QUALIFIED PHYSICIAN AND BY CERTAINTY OF DIAGNOSIS

	Attended by Qualified Physician				*No Qualified Physician*				*Total*			
Year	*Certain*	*Doubtful*	*Unknown*	*Total*	*Certain*	*Doubtful*	*Unknown*	*Total*	*Certain*	*Doubtful*	*Unknown*	*Total*
1957	40	8	0	48	94	42	11	147	134	50	11	195
1958	52	12	0	64	96	45	12	153	148	57	12	217
1959	44	20	0	64	73	52	14	139	117	72	14	203
Total	136	40	0	176	263	139	37	439	399	179	37	615
% of particular class	77	23	0	100	60	32	8	100	65	29	6	100

fied medical attendance in a proportion of 60%; instances of death from traumatic injury, from the common communicable diseases and from neoplasms were usually definite and identified with ease. In all, about two-thirds of cases were within the relatively certain category and one-third doubtful or unknown.

Of doubtful cases, with and without qualified medical care, an identifiable disease was suggestive in about three-fourths of instances, and for one-fourth judgment could extend no further than a symptomatic diagnosis, as of convulsions or fever of undetermined origin.

The unknown cases were of varied nature: persons found dead, notably infants, or those where the attendant was uncooperative or ill-informed. For instance, the response might be "What disease do you need to kill a tiny baby," or "He just got the disease that little babies get," or "We found him dead after we put him to bed," or "He only cried and cried and how can a little baby only a few days old tell about his sickness or how can we know." Leading questions sometimes led to no better response.

The causes of death as determined by survey for a series of 615 fatalities are presented in Table 8. Rates are the number of deaths per year by cause per 100,000 population, the survey population being used. Tuberculosis is well represented, in children and as pulmonary tuberculosis of later life. Among other respiratory infections, pneumonia occupies a prominent place, which, if combined with bronchitis, places this group well up among fatal illnesses.

It is not surprising that infections of the gastrointestinal tract, etiology unspecified, lead all other infections and all causes of death, the rate being 187 per 100,000 population per year. Typhoid fever makes an important contribution. The dysenteries, recognized as such on clinical grounds only, are an inconsequential factor; additional numbers doubtless are included in the first group.

Whooping cough and measles, the two common communicable diseases of childhood having an appreciable fatality, are here significant factors in mortality: in these particular years, measles more than whooping cough in a proportion of about 2 to 1. Tetanus is easily the outstanding feature of the general class of other infectious and parasitic diseases constituting Class B17. The problem centers mainly in tetanus of the newborn; and peculiarly male infants were affected twice as often as females.

Deaths from accidental injury are commonly recorded in the records of chowkidars as due to disease. A feature of this study was the demonstration of their relative frequency in Indian rural life as in most countries of the world. Their position among leading causes of death suffers by the preponderance of deaths from acute infection, but their significance is not to be discounted.

Heart disease and vascular lesions affecting the central nervous system, so commonly high on the list of deaths in Western countries, here rank well down, as do malignant neoplasms. Birth injuries and associated conditions, Class B42, are uncommonly frequent.

The Ten Leading Causes of Death. It is now possible to derive a list of the ten leading causes of death in the village populations from the fatalities identified during the three years, Table 9. Infections of the gastrointestinal tract easily rank first. Pneumonia is not far behind. There is question that the figure is loaded by the presence of terminal pneumonias after other diseases, although knowingly no such instance was included; that would appear more

TABLE 8.—DEATHS, CAUSES OF DEATH AS DETERMINED BY MEDICAL CASE STUDY, AND DEATH RATES BY CAUSE PER 100,000 POPULATION, AMONG RESIDENTS OF ELEVEN NORTHERN INDIAN VILLAGES, JANUARY, 1957, TO DECEMBER, 1959

	Cause of Death	*No. Deaths*	*Deaths per 100,000 Population*
B1	Tuberculosis of respiratory system	33	90.8
B2	Tuberculosis, other forms	7	19.2
B3	Syphilis and its sequelae	1	2.7
B4	Typhoid fever	26	71.6
B5	Cholera	0	—
B6	Dysentery, all forms	8	22.0
B7	Scarlet fever and streptococcal sore throat	0	—
B8	Diphtheria	2	5.5
B9	Whooping cough	8	22.0
B10	Meningococcal infections	1	2.7
B11	Plague	0	—
B12	Acute poliomyelitis	1	2.7
B13	Smallpox	1	2.7
B14	Measles	18	49.5
B15	Typhus and other rickettsial diseases	0	—
B16	Malaria	0	—
B17	All other diseases classified as infective and parasitic	40	110.2
B18	Malignant neoplasms, including neoplasms of lymphatic and hematopoietic tissues	28	77.1
B19	Benign neoplasms and neoplasms of unspecified nature	1	2.7
B20	Diabetes mellitus	1	2.7
B21	Anemias	8	22.0
B22	Vascular lesions affecting central nervous system	9	24.7
B23	Non-meningococcal meningitis	1	2.7
B24	Rheumatic fever	0	—
B25	Chronic rheumatic heart disease	5	13.7
B26	Arteriosclerotic and degenerative heart disease	18	49.5
B27	Other diseases of heart	1	2.7
B28	Hypertension with heart disease	5	13.7
B29	Hypertension without mention of heart	5	13.7
B30	Influenza	0	—
B31	Pneumonias	44	121.1
B32	Bronchitis	18	49.5
B33	Ulcer of stomach and duodenum	0	—
B34	Appendicitis	1	2.7
B35	Intestinal obstruction and hernia	5	13.7
B36	Gastritis, duodenitis, enteritis, and colitis, except diarrhea of the newborn	68	187.2
B37	Cirrhosis of liver	7	19.2
B38	Nephritis and nephrosis	6	16.5
B39	Hyperplasia of prostate	4	11.0
B40	Deliveries and complications of pregnancy, childbirth, and the puerperium	7	19.2
B41	Congenital malformation	8	22.0
B42	Birth injuries, postnatal asphyxia and atelectasis	29	79.8
B43	Infection of the newborn	6	16.5
B44	Other diseases peculiar to early infancy, and immaturity unqualified	45	123.9
B45	Senility without mention of psychosis, ill-defined and unknown causes	64	176.2
B46	All other diseases (Residual)	50	137.7
BE47	Motor vehicle accidents	0	—
BE48	All other accidents	23	63.3
BE49	Suicide and self-inflicted injury	0	—
BE50	Homicide and operations of war	2	5.5
		615	

or less inevitable where dependence was solely on clinical and epidemiological evidence. Tuberculosis in third place is rather lower than would be expected, and might well receive additions from deaths recorded as bronchitis and pneumonia, were better diagnostic facilities available. Tetanus occupies a surprising fourth place. Typhoid fever is definitely an important disease in these village localities; its observed frequency correlates with that of intestinal infection in general and with the state of environmental sanitation.

TABLE 9.—THE 10 LEADING CAUSES OF DEATH IN ELEVEN VILLAGES OF NORTHERN INDIA, AS DETERMINED BY CASE STUDY, JANUARY, 1957, TO DECEMBER, 1959

Cause of Death	*No. Deaths*	*Deaths per 100,000 Population*
Acute diarrheal disease	68	187.2
Pneumonia	44	121.1
Tuberculosis	40	110.2
Tetanus	33	90.8
Heart disease	29	79.8
Birth injuries, *etc.*	29	79.8
Cancer	28	77.1
Typhoid fever	26	71.6
Accidents	23	63.3
Measles	18	49.5

Heart disease and cancer have far less significance than in the Western world with its older populations and the communicable diseases under good control. Chronic asthma with emphysema among elderly men is a common clinical finding reflected in deaths from bronchitis.

Another similar list is limited to deaths seen by physicians. Diagnoses are more reliable, Table 7, but the series is small and selected. The patients seen by a physician tend to have illnesses of longer duration, because of inability of a physician to reach a village with any promptness and for an acutely ill patient to make the trip by bullock cart to a neighboring town and medical care is arduous, time-consuming and of some risk. The series is further selected because costs and other difficulties tend to restrict medical care by a physician to the more important members of the community, rather than a sick child.

The result is a list of ten leading causes of death that differs in order, but not in the diseases themselves, except that dysentery appears. Physicians seemed to prefer that term to the more general gastrointestinal infection of unknown etiology, although no case was confirmed bacteriologically. At any rate, tuberculosis is first in this list, reinforcing the opinion previously expressed of its presumably more important place among total deaths. Cancer ranks second, with gastrointestinal infections third. Typhoid fever and tetanus are still included, as is measles. Pneumonia is fifth.

The leading causes of death as derived from reports of chowkidars have little value. Two-thirds of deaths are attributed to 2 causes, cough and fever, or fever with weakness.

Discussion and Summary. This presentation set out to record some of the facts to be had about death rates in an agrarian community of India; to determine the trends those rates were following; and to identify so far as possible the responsible causes of death. The answers to those questions are fairly definite.

The recorded death rates for the study area do not reflect the true magnitude. The crude death rate is perhaps a fourth higher than reported. The sensitive health index afforded by infant mortality is underestimated still more. The trend of the various death rates shows a highly satisfactory downward course over at least 15 years under the impact of public health programs,

and what has come to be appreciated as an equally important factor, an improved economy and a strengthened social structure. The decline in death rates suggests an increasingly favorable situation so far as health matters are concerned. For the population problem the opposite holds; the future looks more formidable.

Little solid information exists on the diseases responsible for deaths in these rural communities; it is far short of the need in future planning for population dynamics or for public health. The survey shows infections to be the outstanding consideration. So much progress has been made in the control of communicable diseases in many parts of the world that experts are called upon from time to time to remind the medical world that these diseases are still here and not to be lost sight of in the newer and justified enthusiasm about chronic degenerative processes, mental disorders and a variety of others. A look at Table 9 gives sufficient emphasis in this instance.

Perhaps the main generalization from these studies is that the health needs of rural communities are not to be judged by what is known of great metropolitan centers. Rural populations have their individual problems; the same diseases often behave in a different manner in the two situations. There is good explanation for the existing sound body of information about urban communities and the paucity of information about villages; it is harder work to gather information in the countryside and it takes more time to acquire numbers that have significance; it is not so convenient for the investigator commonly interested in these matters, and it is likely to be more costly. There is need for more of the Pickles[24] sort of epidemiology in country practice, or, better still, the organized effort taking form at the University of Iowa in their Institute for Agricultural Medicine[31]. Even in the United States, with all of its investment in public health research and its development of county health departments, this is a unique endeavor.

The strength of the present investigations is in the epidemiological approach, a study of all the deaths that occurred in a community over an appreciable time, with as much attention to the exactness of the population at risk as to the events that occurred. This has material advantages over the selected material available through clinic and hospital studies.

Some of the shortcomings of this study have been stated, particularly in determinations of causes of death where reliance was necessarily on clinical and epidemiological procedures. Workers made no secret of an occasional heartfelt wish for just one necropsy. There was universal appreciation of the challenging experience that would follow could only the full facilities of a modern medical center be brought to the task.

On the other hand, the philosophy of Thoreau also has much force, that the place to learn about Nature is where it is found. The basic elements of deaths as seen in a rural agrarian society have their value; that has been the present objective. Future publications will deal with demographic characteristics of the persons who died and the influence exerted on population increase; with the kind of medical care they received in their last illness; with the special problem of tetanus; and with the causes of deaths at various ages and for the two sexes with special consideration of infant and child mortality.

Such knowledge is indispensable in framing future remedial schemes, either in amelioration of the population problem or for the promotion of the public health. Its absence can lead to the acceptance and prolongation of mistaken or outmoded practices.

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PATHOLOGY AND BACTERIOLOGY

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RICKETTSIAL AND VIRAL TOXINS

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ALTHOUGH bacterial toxins have been known since the early years of microbiology, it was not until 1940 that experimental evidence was presented indicating that a member of the *Rickettsiaceae* had an associated toxin[55] and in 1942, the existence of a viral toxin was first noted in studies with Newcastle disease virus[27]. The definition and characterization of viral and rickettsial toxins has been more difficult than with toxins elaborated by bacteria. The primary difficulty has been one of separation of the toxic principle from the infectious particle. The lability of the toxins when subjected to various physical and chemical agents has made the problem still more complex.

Burnet[28] has defined toxicity with respect to viruses as "the production of lesions in the absence of evidence of multiplication adequate to account for them." Although the use of the term viral toxin to describe the phenomena which fit Burnet's definition has been criticized for various reasons, it will be used in the present review since no other term so readily describes the events which occur in the absence of true viral reproduction. The definition applies equally well to the toxins associated with the *Rickettsiaceae*.

RICKETTSIAL TOXINS. The *Rickettsiaceae* occupy a position somewhere in between the bacteria and large viruses of the psittacosis-lymphogranuloma group and, although they are primarily parasites of arthropods, certain species can cause disease in man and lower animals.

Although the toxic appearance of patients during the acute phase of certain rickettsial diseases was suggestive of associated toxins, it was not until 1940 that experimental evidence of the existence of rickettsial toxins was reported. Gildemeister and Haagen[55], working with *Rickettsia mooseri (R. typhi)*, the causative agent of murine typhus, found that mice injected intraperitoneally with heavily infected yolk sac suspensions of the organism died within 4 to 20 hours. There was no evidence that the rickettsiae had multiplied significantly during this time and, in those mice which survived the first 20 hours, actual infection developed a few days later. The early deaths were attributed to a toxin. The toxin was very labile; either heating to 60° C. or storage for

several days at 2° C. destroyed its activity. The addition of formalin also resulted in a loss of toxic activity. The toxin was neutralized by specific immune serum but not by normal serum.

Shortly thereafter the initial findings of Gildemeister and Haagen[55] were confirmed and extended. It was shown that *R. prowazekii,* the causative agent of epidemic typhus, produced a toxin similar to that described for *R. mooseri*[14,98,124]. The Giroud skin test[58], in which the intracutaneous inoculation of either *R. prowazekii* or *R. mooseri* into rabbits results in a skin reaction consisting of erythema followed by an indurated inflamed nodule with central necrosis in 2 to 3 days, was ascribed to the respective toxins by Kligler and Oleinik[98]. Non-infectious preparations were obtained by freezing and thawing the rickettsial suspensions seven to eight times and the authors demonstrated that although such preparations were non-infectious by both egg and animal inoculation they still elicited the typical skin reaction. Bengtson, Topping and Henderson[13,14,78,159,160] studied the toxins of *R. prowazekii* and *R. mooseri* in some detail and reported that the toxins were "toxoided" by the addition of formalin and described methods by which the toxins could be standardized. The specificity of antitoxin against either *R. mooseri* or *R. prowazekii* was reported by several workers[39,63,71], and Hamilton[71] showed that the toxin associated with *R. mooseri* was antigenically distinct from that of *R. prowazekii.* The toxins of different strains of either *R. mooseri* or *R. prowazekii* were not, however, distinct from one another in this respect.

Smadel *et al.*[149] demonstrated that there was a toxin associated with the Gilliam strain of *R. orientalis (R. tsutsugamushi),* the etiological agent of scrub typhus. The toxin killed mice, usually in 3 hours after intravenous inoculation of a heavily infected yolk sac suspension of the organism. For the first hour to an hour and a half after the injection, the mice appeared normal, but then the animals developed obvious dyspnea and weakness, followed by prostration, cyanosis, convulsions and death. Mice which received sublethal doses of toxin recovered from the early toxic symptoms and subsequently died in 4 to 6 days from infection. *R. orientalis* was found in the spleen and lungs of the latter mice. The toxic effects were neutralized by homologous antiserum but not by heterologous antisera. Antisera to heterologous strains of *R. tsutsugamushi* varied in their ability to neutralize the toxin[149]. The observation of Clarke and Fox[36] that hemolytic activity is associated with certain of the typhus rickettsiae has also made possible *in vitro* studies of rickettsial toxins.

In the spotted fever group of rickettsiae, Bell and Pickens[11] have shown that there is a toxin associated with *R. rickettsii* and *R. conorii* but no toxin has been demonstrated in either *R. akari* or *R. australis.* In order to obtain reproducible toxic preparations of the organisms in this group, Bell and Pickens found that it was necessary to continue incubation of the embryonated eggs, in which the rickettsiae were grown, for an additional 36 hours following the death of the embryo. The toxin was tested by intravenous inoculation of the yolk sac suspension into mice and, with positive suspensions, a similar sequence of events to that obtained with other toxic rickettsial preparations was observed[11]. Recently, Bell and Stoenner[12] have reported further on the differentiation of this group of rickettsiae by means of the toxin neutralization test in mice and showed that *R. rickettsii, R. conorii* and the

rickettsia of Siberian tick fever could be differentiated by this test. The rickettsiae of boutonneuse fever, South African tick bite fever, Kenya tick typhus and the Indian tick typhus, on the other hand, could not.

Various workers have made reasonably detailed studies of the properties and activities of the rickettsial toxins. Hemolytic activity lends itself particularly well to *in vitro* studies. Clarke and Fox[36] noted that a hemolysin active against sheep and rabbit but not against mouse erythrocytes is associated with both *R. prowazekii* and *R. typhi*. Such activity could not be demonstrated in suspensions of *R. orientalis*. Hemolytic activity was not separable from the organisms and was inactivated by 0.5% formalin which also destroyed infectivity. The hemolysin was shown to be very heat labile, being progressively inactivated after a few hours at 35° C. or even at room temperature and rapidly inactivated at 56° C. Storage for one to 2 days at 4° C. also brought about a loss of activity. Hemolytic titers were never high averaging between 1:32 and 1:128; there was a close parallel between hemolytic activity and *in vivo* toxic and infectious titers. The reactions were neutralized by homologous immune serum. The mechanism of hemolysis was not defined; the reaction proceeded slowly and never went to completion; that is, no matter what concentration of erythrocytes was used, complete lysis was not obtained. Snyder *et al.*[150] reported that a high concentration of sheep erythrocytes was required for sensitivity, a 25% suspension giving the most reliable results. The hemolytic titrations were incubated at 34° C. for 2½ hours and the released hemolysin measured colorimetrically[5, 150].

The hemolytic reaction requires the presence of glutamate and magnesium ions, possibly calcium and manganese, and is inhibited by such antimetabolites as fluoride, cyanide, azide and iodoacetate[150]. It is of interest in this respect to note that glutamate is a substrate known to be rapidly metabolized by partially purified suspensions of both *R. prowazekii* and *R. mooseri*[23]. When suspensions of *R. prowazekii* are incubated at 36° C. for several hours in the absence of any substrate, the hemolytic and toxic activities are lost[22]. If, however, glutamate, pyruvate or adenosine triphosphate (ATP) are incubated with the organisms, there is no loss of the two properties. When suspensions which have lost their hemolytic and toxic properties are incubated further at 30° C. with added glutamate, there is a partial, and sometimes complete, recovery of these properties. Although no other substrate would seem to have this effect, the addition of ATP or adenylic acid with glutamate results in a more complete recovery. Bovarnick and Allen[22] have shown that during starvation of the rickettsial suspension the concentration of ATP falls to zero but rises on the addition of glutamate. Maximum restitution of hemolytic activity occurred at 30° C. rather than 35° C. despite the fact that more active oxygen uptake and phosphorylation occurs at 35° C.; from this it was concluded that there was some process in addition to phosphorylation required for recovery of the toxic and hemolytic properties[22].

Controlled irradiation of partially purified suspensions of *R. prowazekii* with ultraviolet light reduces the infectivity of the suspensions for embryonated eggs and cotton rats 10^4 and 10^5-fold without a marked loss in toxicity for mice, hemolytic activity or respiration. More prolonged irradiation results in a gradual loss of the latter properties, toxicity for the mouse disappearing first, followed by hemo-

lytic activity and, finally, respiration[5].

Detailed *in vivo* studies of the toxins of *R. prowazekii* and *R. mooseri* have been done in white mice, white rats and rabbits[61,62,116,117,126,127,169,170]. The effects of the toxin in mice and rats have been found to be quite distinct from those in rabbits primarily because of the sensitivity of rabbit erythrocytes to the hemolysin[36,117,150].

White rats and white mice would appear to be equally susceptible to *R. prowazekii* toxin, both species having essentially the same LD_{50} on a unit weight basis. The rat, however, is much more resistant to *R. mooseri* toxin than is the mouse, requiring some ten times more toxin on a unit weight basis for a lethal effect[116]. With both mice and rats there is a latent period of at least 30 minutes, following the intravenous injection of toxin, during which no effects are observed. After the latent period both species exhibit such typical signs of rickettsial toxemia as dyspnea, cyanosis, and prostration. In rats, hyperirritability and convulsive movements which are characteristic of the toxemia in mice are not seen. Death occurs in from 1½ to 4 hours with larger doses of toxin and in up to 18 hours with toxic doses approaching one LD_{50}[116,117,127].

The gross pathological changes seen at necropsy in rats appear to be primarily in the small intestine, and are comparable to those found with bacterial endotoxins[15,30,174]. The visceral blood vessels in general and particularly in the small intestine are dilated and engorged with blood[116,126]. After doses of toxin which caused death in 3 to 4 hours, the mid-portion of the small intestine was intensely congested and often hemorrhagic. Similar but less pronounced changes are seen in the small intestine of mice, with hemorrhage rarely encountered. Microscopically, Parker and Neva[126] found, in addition, that after sublethal toxic doses of suspensions of *R. prowazekii* hepatic lesions in the form of a focal necrosis occurred. These lesions were present at 24 hours and were most prominent in 3 days with healing occurring in 5 days. The hepatic lesions, which are consistent with a toxic etiology, were shown by titrations for viable organisms in the tissues to be caused by the toxin. Moreover, it is known that multiplication of *R. prowazekii* does not occur in mice[116]. With *R. mooseri*, where multiplication does occur in addition to the focal necrosis, there was damage to the Kupffer cells[126].

The physiological changes occurring after the intravenous inoculation of suspensions of *R. prowazekii* and *R. mooseri* have been studied in some detail. It has been reported[36,117] that hemoconcentration occurs in both mice and rats. Neva and Snyder[117] showed, by testing periodic blood samples taken after the intravenous inoculation of toxic typhus rickettsial suspensions, that there was a progressive increase in whole blood specific gravity and a rise in hematocrit values. The specific gravity of the plasma did not change significantly and it was concluded, therefore, that the hemoconcentration was due to the loss of whole plasma rather than to the loss of water alone. Although Parker and Neva[126] found little evidence of acute loss of plasma into the interstitial spaces with the exception of intraluminal escape of blood in the small intestine, Wattenberg *et al.*[170], using the tracers, dextran and azoprotein T-1824, showed that plasma leakage was quite widespread throughout the tissues of mice. The tracers were found in the cardiac and the skeletal muscles in small pools, often perivascular or perineural in distribution. They were also seen in the pancreas, subcutaneous tissue, and in-

testines but not in the brain[170]. Blood pressure readings obtained by cannulization in unanesthetized rats remained normal after intravenous injection of toxin until just prior to death when there was a sudden drop to very low levels. The blood pressure, therefore, remained normal until hemoconcentration was quite advanced and indicated that the animals were able to adjust to, or to compensate for, the marked plasma loss until a critical stage was reached[117].

Wattenberg *et al.*[170], using a transparent chamber[4] attached to a dorsal fold of the skin of the mouse, and more recently Greisman and Wisseman[62], adapting a similar technique to the mesoappendix of rats, have further defined the changes occurring after the intravenous injection of toxic *R. typhi* suspensions. The experimental procedures allowed for continuous microscopic observation of the small blood vessels including the capillaries which supply the respective areas. Careful control studies were done to ascertain that any effects seen were not caused by the techniques used. After the first 20 to 30 minutes following the intravenous injection of the rickettsial suspension, during which no visible changes occurred, there was a progressive slowing of the blood flow and constriction of the arterial vessels, but no change in the size of venules and veins. As the blood flow slowed, edema of the subcutaneous tissues occurred, reverse flow was common in the venous channels and although there was no obvious sludging or increased stickiness of the cellular components, the blood column tended to segment. As the reaction progressed the tissues took on a ground glass appearance and the increase in interstitial fluid made observation of the smaller vessels increasingly difficult[62,170]. Microhematocrit determinations showed hemoconcentration as had been found earlier[117], and cortisone, known to affect vascular permeability, had no effect on either the time or intensity of the reactions[62].

Greisman and Wisseman[62] compare the effects of the toxin of *R. mooseri* with the effects of the endotoxin of *Salmonella typhosa* and with those of hemorrhage. It is of interest to note the effect of epinephrine obtained by the investigators in the three conditions. In fatal rickettsial toxemia the reactivity of vessels of the mesoappendix remained unchanged, whereas during hemorrhage there was a 30 to 100-fold increase in reactivity and with *S. typhosa* a marked change in reactivity depending in degree on the dose of toxin[62].

A histochemical survey of mice inoculated intravenously with toxic suspensions of *R. typhi* has failed to show any effect of the toxin on the activity of such enzymes as succinate, lactate, α glycerophosphate and triose phosphate dehydrogenases, peroxidase, alkaline phosphatase, adenosine-5-phosphatase, and myristoylcholine esterase[169]. A late and nonspecific decrease occurred in the ability of the liver to reduce neotetrazolium chloride anaerobically in the absence of added substrate. Since this was a terminal effect occurring when vascular permeability and hemoconcentration were known to be advanced[117], it was felt to be a secondary effect and not related to the primary action of the toxin[169].

As an explanation of the physiological effects of rickettsial toxins, Neva and Snyder[117] have suggested that there is direct cellular injury, partly because the toxic effect could not be passively transferred. Greisman and Wisseman[62] pointed out that, since an increase in vascular permeability occurred with rickettsial toxemia in rats and mice with no change in reactivity to epinephrin, the reaction must be of a

specific and unusual type and possibly confined to the endothelium[62].

Studies done on the effect of *R. typhi* toxic suspensions in the rabbit have presented an entirely different picture from those done in white mice and white rats[127]. The difference is due to the susceptibility of rabbit erythrocytes to the associated hemolysin, as discussed previously[36]. Patterson, Wisseman and Smadel[127] attached a polyethylene catheter to the femoral artery of the rabbit set up in such a way that simultaneous electrocardiograms and arterial blood pressure readings could be recorded and serial blood samples taken at precise intervals following the intravenous injection of the toxin. Death occurred in rabbits within one to 2 hours with four LD_{50} of toxin, and with smaller doses (one to two LD_{50}) death might ensue in any period of from one to 4 hours. Rabbits dying rapidly from the effects of the toxin appeared normal for 10 to 15 minutes following which they became weak, restless and prostrate and died with terminal generalized convulsions. Hemoglobinuria occurred and preliminary observations showed that hemolysis initiated *in vivo* by toxin continued *in vitro* at room temperature for approximately one hour. Consequently precautions were taken to minimize the *in vitro* effects by chilling of the syringes and separating the plasma from the cells at 4° C.[127]. Rabbits which were bled one to 2 hours after administration of the toxin showed a high concentration of hemoglobin in the plasma, ranging from approximately 162 mg. per 100 ml. in those that survived, to as much as 1136 mg. per 100 ml. in those that died. Control yolk sac inoculation had no effect. The hemoglobinemia was neutralized by specific immune serum or by heating the toxic suspensions prior to inoculation[127]. Hyperkaliemia also occurred, the rise in potassium paralleling the rise in plasma hemoglobin except in those cases where death was delayed until 4 hours, when the rise occurred abruptly just prior to death. A rise in plasma phosphates and a rise in blood urea nitrogen indicated renal failure and suggested to Paterson *et al.*[127] that the potassium accumulation was a result of renal dysfunction. It was felt that the accumulated potassium was not only from the lysed erythrocytes but also from damaged tissue cells. Electrocardiographic abnormalities occurred with marked bradycardia approaching actual cardiac standstill just prior to respiratory failure. There was a progressive fall in blood pressure from 80 mm. of mercury to about 33. Hemoconcentration did not occur and, in actuality, there was a slight but consistent decrease in whole blood specific gravity which was present also in control animals and was probably the result of the frequent sampling of blood. It was found that in those rabbits which died rapidly (within 90 minutes) that 30% of the erythrocytes were lysed during the first 30 minutes. Whole blood hemoglobin decreased within a half to one hour before death and was lost apparently to the urine and interstitial spaces[127]. An initial leukopenia was followed by leukocytosis, a phenomenon which has been observed with bacterial endotoxins[15,30,174].

Neither ACTH nor cortisone has any effect on rickettsial toxemia[91]. Because of loss of fluids during rickettsial toxemia in mice and rats and because of enhanced susceptibility of mice to the toxin when they were deprived of water, a fluid replacement therapy was attempted by Neva and Snyder[117] with no effect, nor did antihistamines result in any alleviation[117]. Cox[37,38] pointed out that patients with toxic symptoms from rickettsial infection showed mark-

ed clinical improvement within a few hours after the administration of aureomycin. It was shown that aureomycin had a direct neutralizing effect on certain rickettsial toxins *in vitro* and that, if the toxin and aureomycin were mixed and held 20 to 30 seconds before intravenous injection into mice, the mice survived while control animals died. There was no rickettsiocidal action since the non-toxic mixtures had the same LD_{50} for embryonated eggs as toxic mixtures. Karp and Snyder[90], however, reported that aureomycin and terramycin inhibited the respiration of purified suspensions of *R. prowazekii* and *R. mooseri in vitro* and that the inhibition of respiration was correlated with a decrease in *both* toxicity and infectivity for white mice. Chloramphenicol was not so effective as aureomycin and terramycin but inhibited toxicity more than respiration. It would be of interest to determine the effect of the toxin neutralizing substance produced by *Penicillium cyaneofulvum*[115] on the toxins of *R. prowazekii* and *R. typhi.*

In summary, it is evident that *R. prowazekii* and *R. typhi (R. mooseri)* as well as certain other rickettsiae have two distinct *in vivo* toxic effects which may be demonstrated in different experimental animals. In white mice and white rats the toxin causes capillary damage with plasma loss to the tissues resulting in hemoconcentration and eventual death from vascular collapse. In rabbits, the hemolysin plays a definite role causing *in vivo* lysis of erythrocytes and hypotension occurs without capillary damage or hemoconcentration. *In vitro,* only the hemolysin may be demonstrated.

VIRAL TOXINS. *1. Toxins of the Psittacosis-lymphogranuloma group.* The possibility of a toxin associated with lymphogranuloma venereum virus was suggested from certain clinical symptoms observed in severe generalized lymphogranuloma venereum[76,77]. Moreover, in experimental studies, it was shown that chick embryos died after yolk sac infection, even though the embryo was not invaded by the virus to any appreciable extent[138], and convulsive deaths which occurred in mice after intracerebral inoculation of the virus could not be explained entirely on the basis of the lesions found[139].

In 1943 Rake and Jones[139] demonstrated the presence of a toxin associated with lymphogranuloma venereum virus in heavily infected yolk sacs of moribund embryos. The toxin disappeared if the eggs were incubated after death of the embryo. The toxin is lethal for mice after intravenous or intraperitoneal inoculation, although a larger dose is required by the latter route[139]. Most mice die within 24 hours after the intravenous inoculation of 0.5 ml. of a yolk sac suspension of the virus, with some mice dying as early as 4 hours. Convulsions often precede death, especially in those animals which die early. Certain tissues, notably the liver, show evidence of toxic damage in the form of focal necrosis. Focal lesions in the brain and small areas of edema and hemorrhage in the lung and kidneys are also found. The rapidity of the reaction, the lack of evidence of any infectious process, and the nature of the lesions in the liver led the authors to the conclusion that a toxin was responsible[139].

The toxin is not separable from the elementary bodies of lymphogranuloma venereum virus; it can be neutralized by homologous antiserum either by prior *in vitro* mixing with the viral suspension or prior inoculation of the antiserum into the test animal[139].

Toxins have also been demonstrated in suspensions of meningopneumonitis and mouse pneumonitis viruses[140]. Such toxins are all labile and not sep-

arable from their respective elementary bodies. The toxic minimal lethal dose, in terms of infective units for eggs, is very high corresponding to approximately 36 million such units. The characteristic lesion found in all mice except those which die very rapidly is focal necrosis of the liver. Hamre and Rake[72] have reported further on the pathological findings following toxemic death with viruses of this group. Despite the fact that necrosis of liver cells was not so pronounced as described previously[137], there was a diffuse cloudy swelling with occasional cells showing frank necrosis[72]. In those instances where death was very rapid, as with the toxin of mouse pneumonitis virus which killed mice in one to 4 hours, either no liver lesions were seen or they were much reduced. The toxins are inactivated by formalin but their antigenicity remains intact; neutralization reactions are highly specific. For example, Manire and Meyer[106] have studied 39 strains in this group and have found that 29 of the strains produced toxins which fell into six separate specific groups on the basis of toxin neutralization tests.

Hamre and Rake[72] have shown that feline pneumonitis virus also possesses a toxin and they were the first to call attention to the diphasic death curves which occur and which have been confirmed by Manire and Meyer[104,105]. With feline pneumonitis virus, for example, there is a toxic death peak during the first 24 hours followed, on the third day, by a second peak caused by infection. Similar curves, varying slightly in timing, are found with meningopneumonitis and mouse pneumonitis viruses. Lymphogranuloma venereum virus on the other hand shows only the first peak, since it does not infect mice by the intravenous route[72].

Rake and Hamre[137] studied the effect of sulphonamide on the toxins of the psittacosis-lymphogranuloma viruses. Sulphamerazine has no effect on any of the toxins but does prevent late infectious deaths with mouse pneumonitis virus. Feline and meningopneumonitis viruses, insensitive to sulphamerazine, show both toxic and infectious deaths. With lymphogranuloma venereum virus, where no infection occurs after intravenous inoculation, there is no effect on the toxin even when the toxin is used in very small doses. Manire and Meyer[105] found similar results when testing the effect of aureomycin and penicillin on these viruses. With certain strains which did not exhibit a diphasic death curve, it was shown that toxic deaths did occur during the first 24 hours followed very closely by infectious deaths. The infectious deaths, of course, did not occur in the treated animals. It was of interest that most strains which showed a diphasic death curve were strains established in mammalian species (that is, avian species did not exhibit the diphasic death curve)[104,105].

Similar toxins have been demonstrated with various other members of this group of viruses, isolated from such diverse sources as snowy egrets[143], opossums[141], calves[177,178] and turkeys[53,110]. These toxins, however, have not been investigated in detail.

2. *Toxins of the small viruses.* a. *Influenza virus.* The demonstration of a toxin associated with influenza virus came in 1944 when Henle and Henle[81] reported that the intracerebral inoculation of either high titered or concentrated influenza virus caused death of mice in 12 to 48 hours without multiplication of the virus. This observation has been confirmed by other workers[65, 70,165] and studied in greater detail in subsequent work by the Henle's[82,83,84, 85]. Mice injected intracerebrally with non-neurotropic influenza virus react first with marked hyperirritability fol-

lowed by tremor and clonic convulsions and, in most instances, death[81]. The reaction is enhanced if the mice are suspended by their tails and twirled several times; survivors tend to be spastic for a few minutes and refractory to a second attack for a short period. Controls of normal allantoic fluid, broth diluent or chorioallantoic membrane particles are without effect, indicating that the reaction is not caused by either the suspending fluid or the activation of a latent neurotropic virus. The reaction is elicited by a large number of influenza A virus strains as PR8, WS and F-99 and the Lee strain of influenza B virus which shows that it is a characteristic property of influenza virus as a species. Recently, Seto *et al.*[148] compared the neurotoxicity of five Asian influenza virus isolates with both the PR8 and FM1 strains of influenza virus. It was found that Asian influenza viruses were able to induce toxic reactions after intracerebral inoculation into mice and that in terms of hemagglutinating units necessary to elicit the reaction the Asian isolates were comparable to the other influenza virus strains. The reaction is neutralized by specific immune serum with no cross immunity between influenza A and B viruses. The virus can not be passed from mouse brain to mouse brain and, in fact, virus decreases in concentration in the brain to less than 0.1% of the amount found immediately following inoculation[81].

Histological studies, made after intracerebral inoculation of the virus, show destruction of the ependymal lining of the ventricles as the predominant lesion[81,82]. In a study with both a neurotropic and a non-neurotropic strain of influenza A virus using a fluorescent antibody, Fraser *et al.*[51] reported that with infant mice the non-neurotropic strain (MEL) did not progress beyond the leptomeninges, ependyma, choroidal epithelium and in the parenchyma, whereas the neurotropic strains (NWS, N-MEL) spread into nerve tissue and fluorescent staining was generalized throughout the brain.

The toxic property of influenza virus is inseparable from the virus particle on high speed centrifugation, by adsorption onto, and elution from, chicken red blood cells or by precipitation with protamine; it is quite labile, being destroyed by ultraviolet irradiation, by heat at 56° C. for 15 minutes or 37° C. for 48 hours and by the addition of formalin[82,84]. It has been shown, however, that the infectious and toxic properties may be differentiated, since the ability of influenza virus to propagate is lost at a faster rate when viral preparations are irradiated, heated or formalinized[83,85].

Toxic reactions following intracerebral inoculation of influenza virus are demonstrable not only in different strains of mice but also in rats, hamsters, guinea pigs and ferrets[82]. Influenza virus is also toxic when injected intravenously or intraperitoneally into mice, rats, rabbits and guinea pigs[83,84,94]. In mice, death occurs within 16 to 72 hours with no evidence of viral multiplication nor can the virus be transferred, in suspensions of various organs, from mouse to mouse. At necropsy mice show engorgement of the blood vessels of extensive portions of the small intestine with marked edema and distension of the gut. The lumen of the duodenum, jejunum and ilium contains bloody mucous material varying from pink to dark red. In the early stages of the toxemia the lungs are normal but, in some instances at least, a pleural exudate containing lymphocytes appears later. Microscopically, the liver and spleen are hyperemic and the liver diffusely necrotic[83]. It

appears, however, that mice are five to ten times more susceptible to the toxin of influenza virus when it is inoculated intracerebrally than when it is given intravenously[103].

A comparison between the infectious, hemagglutinating and toxic titers of different influenza virus preparations has shown that those preparations with the higher infectious and hemagglutination titers are also the more toxic ones[82,83,148]. Such preparations may be obtained within 48 hours' incubation in the allantoic fluid of the embryonated hens' egg when highly diluted inocula are used[82,83]. Toxicity is demonstrable only when active influenza virus is injected and, if an irradiated preparation is inoculated prior to active virus, there is evidence of interference[81].

The toxicity of influenza virus may be demonstrated also in the eye of the rabbit[44,173]. In this case corneal opacity occurs in one to 3 days following inoculation of influenza A virus. Rapidity of the reaction appears to depend on the potency of the virus preparation used. Wilcox *et al.*[173] found that the reaction was associated with a progressive destruction of the corneal endothelium. There was no evidence of viral multiplication either by infectivity or hemagglutination titrations. By means of human albumin labelled with I_{131} and inoculated after the toxic reaction had developed, it was shown that the endothelial changes resulted in an altered exchange relationship between the corneal stroma and the aqueous humor resulting in corneal edema and opacity. Histological studies showed that the posterior surface of the cornea was devoid of endothelial cells and appeared to consist of Descemet's membrane littered with debris and leukocytes. Regeneration of the epithelial cells followed by recovery from corneal edema and opacity occurred in certain cases depending on the dose of virus used[173].

The CAM strain of influenza A virus was studied by Sugg[155,156,157] who found that intranasal instillation of large doses of egg-adapted virus into mice resulted in death with little, if any, evidence of viral multiplication. Death was felt to be due to the toxin, since the effect was not transmissible from mouse to mouse, using lung suspensions prepared from the dead mice. The pulmonary lesions caused by the toxin appeared the same as those caused by infection. Either mouse-adapted or non-mouse-adapted virus was capable of killing mice. Sugg stated that death in both cases was by the action of toxin. With mouse-adapted virus larger inocula killed mice rapidly without significant multiplication by virtue of the preformed toxin, while smaller inocula required a longer period of time to prove fatal, since multiplication to a critical concentration was necessary to obtain a sufficiently high level of viral toxin[157]. In support of this theory, the microbial product, xerosin, which does not affect the infectious property of influenza virus has been shown to suppress the pulmonary lesions associated with influenza virus and also to protect mice from the effects of intracerebral inoculation of influenza virus toxin[65,67,68].

Studies with culture filtrates of *Vibro cholerae (V. comma)* have been done in conjunction with the investigation of influenza virus toxin. The culture filtrates contain an enzyme known as receptor-destroying enzyme or RDE which destroys the virus receptors both *in vitro* (on erythrocytes) and *in vivo* (mouse lung tissue)[152,153,154]. RDE prevents the intracerebral effects of the viral toxin and it was suggested that the effect was due to the blocking of the attachment sites for the virus[166,167]. Subsequent work, however, has shown

that heat inactivated RDE, no longer effective as a blocking agent, is still active in its capacity to protect mice from the toxic effects of influenza virus[66].

Influenza virus has yet another toxic effect; that is, its pyrogenic activity. Wagner *et al.*[167] have shown that the intravenous injection of influenza A or B viruses into rabbits results in a temperature rise of some 2.5 to 3.5° F. which becomes manifest after a latent period of about 2 hours. A lymphocytopenia occurs in conjunction with the pyrogenic effect which has been noted also by other investigators[42,52,75]. Tolerance to both effects is observed when a second injection is given the following day and may persist for as long as 11 days[16]. RDE diminishes the febrile response to influenza virus, although near lethal amounts of RDE are required. It might be expected that the general debilitating effect caused by such large doses of RDE might suppress the febrile response in its own right, but that this is not so, is illustrated by the fact that *Salmonella typhosa* vaccine still produces its usual febrile response under the same conditions[166]. This also suggests that the pyrogenic activity of the viral toxin is based on some mechanism different from that of the bacterial endotoxin.

The factor responsible for the pyrogenic effect is more stable to heat than the infectious property of influenza virus but it is destroyed if the viral preparation is heated to a point where hemagglutinating activity disappears[167]. The viral pyrogen, however, is less heat stable than bacterial endotoxins which can, of course, withstand autoclaving[15,30,174].

Atkins and Hueng[8,9,10] reported that, after the intravenous inoculation of influenza virus into rabbits, a substance with pyrogenic properties appeared in the blood stream of the rabbits. This substance, endogenous pyrogen, can be passively transferred to normal rabbits and its titer determined by measuring the pyrogenic response. The titer of the circulating pyrogen is closely correlated with the fever produced by the virus. Rabbits which are tolerant to a second dose of virus fail to show the appearance of endogenous pyrogen. Such animals do, however, react with fever to the passive transfer of endogenous pyrogen from a reactive animal. Endogenous pyrogen is more stable to heat than the pyrogenic factor of the virus *per se* and exhibits a shorter latent period upon transfer to recipient animals. The virus-induced endogenous pyrogen when transferred to either normal or tolerant recipients does not produce significant changes in the leukocyte counts, although the injection of the virus itself does cause a lymphocytopenia[9]. A lymphocytopenia does occur, on the other hand, following the injection of both bacterial endotoxin[15,30,174] and tissue polysaccharides[99], indicating that endogenous pyrogen differs from both these substances.

Studies on the reaction of influenza virus with guinea pig polymorphonuclear leukocytes by Ginsberg and his coworkers[48,49,57] have indicated that the virus inhibits the ability of the leukocytes to phagocytose bacteria, and this may be a toxic effect since no detectable viral multiplication occurs. It has been noted that influenza virus also inhibits anaerobic glycolysis of polymorphonuclear leukocytes when glucose is used as the substrate[48]. Calcium was required for this inhibitory effect and it occurred with glucose-6-phosphate but not with fructose-6-phosphate as the substrate. The results suggest that the inhibition is probably due to a reduction in phosphohexoisomerase activity[49]. Phagocytosis by the leukocytes was increased when

glucose or monophosphorylated hexoses were added. When either glucose or glucose-6-phosphate was present, influenza virus inhibited the phagocytic activity. If, however, fructose-6-phosphate was added, influenza virus could no longer inhibit phagocytosis. It was suggested that inhibition of phagocytosis was probably through the same metabolic block as the inhibition of glycolysis[49].

The possible role of influenza virus toxin in the pathogenesis of influenza in man was suggested first by Henle and Henle[83]. There are numerous reports in the literature of neurological and cardiac disturbances during the course of influenza in man which might very well be of toxic origin[19,21,47,87,88,89,107,123,125,158,168]. Influenza is generally a relatively mild disorder and, as a result, pathological studies are not available in the large majority of cases. However, in certain instances where they have been done as, for example, those reported by Finland *et al.*[47], the findings have suggested that certain changes might be the result of toxic action rather than infection *per se*. In 2 cases there was an associated myocarditis which resembled most closely that found with diphtheria toxin. There was necrosis of occasional muscle fibers and moderate cellular infiltration of the interstitial tissues. Again in a series of 30 cases of Asian influenza, Walsh *et al.*[168] reported that 75% of their patients had digital vasoconstriction and mild electrocardiogram irregularities at the height of the disease which disappeared with convalescence. In this respect the experimental work of Kempf and Chang[33,93] should be mentioned. In a study of the hypotensive effect of influenza virus in rats, it was shown that there is a very rapid fall in blood pressure immediately after the injection of influenza virus. The hypotensive factor apparently acts directly on the myocardium, resulting in a marked reduction in the amplitude but not the rate of the ventricular contractions. By observation of the heart *in situ* it was found that 2 minutes after the injection of the virus the ventricles become dilated and the auricles and great veins engorged with blood. Vagotomy ruled out reflex depression of the myocardium as the cause of the hypotensive effect. Microscopically the only abnormal finding was engorgement of the myocardial vasculature. In controls, normal allantoic fluid was without effect. The hypotensive factor associated with influenza virus is very heat stable, 16 hours at 56° C. being required to destroy it. The effect is neutralized by specific immune serum[33,93].

In conclusion, the findings of Schlesinger[147] and Henle *et al.*[80] should be mentioned. Schlesinger[147] has shown that non-neurotropic influenza virus when inoculated intracerebrally into mice induces an increase in hemagglutinins and complement-fixing antigens in spite of a progressive decrease in infectious titer. The hemagglutinin and complement-fixing antigen titers remain at a level consistent with a single rise period for at least 48 hours. It was stated that the "incomplete" influenza virus was formed in a single cycle and that the yield of incomplete virus was proportional to the amount of infectious inoculum. It was felt that either complete or incomplete virus could impair the integrity of the cell and that, in reality, the toxicity of influenza virus was a manifestation of infection. Similar results have been demonstrated by Henle *et al.*[80] with cultures of HeLa cells; that is, a rise in hemagglutinins and complement fixing antigens without the formation of infectious virus. Even if the formation of incomplete virus should be a manifestation of infection it is still

obvious that we are not dealing with infection in the usual sense of the term. On the other hand, the studies of Henle and Henle[83,85] on the effects of ultraviolet irradiation of influenza virus suspensions strongly indicate that the infectious and toxic properties are two separate entities. It is, of course, known that both the complement fixing antigens and the hemagglutinins appear prior to the infectious virus when the virus is grown in embryonated eggs[86] and that non-infectious virus or incomplete virus is produced when the virus is passed undiluted in embryonated eggs[102]. Such incomplete virus when concentrated and injected intracerebrally into mice has a very low toxicity compared to fully active virus with the same hemagglutination titer[103,109].

Recent studies by Paucker *et al.*[128] and Birch-Anderson and Paucker[18] suggest that incomplete or non-infectious hemagglutinins of influenza virus are lacking in ribonucleic acid which, presumably, prevents their replication. The production of such particles certainly does not constitute infection in the true sense of the term.

b. *Newcastle Disease Virus.* Newcastle disease virus (NDV) resembles influenza virus in certain ways such as the elaboration of hemagglutinins and toxins, although they are not serologically related[27]. Intranasal instillation of NDV followed 24 hours later by either a second dose of virus or of saline was found by Burnet[27] to result in lung consolidation with little evidence of viral multiplication. Passage from mouse to mouse was not possible. Other workers[40,73] have confirmed these findings which recall the experiments with the CAM strain of influenza A virus[155,157]. NDV also causes central nervous system disturbances in mice following intracerebral inoculation without evidence of multiplication[64,163]. The age of the mice used has been found to influence the outcome of intracerebral injection[162,163,164]. Suckling mice are not affected by the toxin even though proliferation of the virus occurs[162]. Weanling and older mice, despite the fact that they do not support multiplication of the virus, are susceptible to its toxin[164]. Mice which die after intracerebral inoculation without multiplication of NDV exhibit, in many cases, spastic paralysis. Less toxic strains result only in transient incoordination, ruffling of hair and apparent anorexia. The characteristics of infection differ from those of toxicity; paralysis, for example, being very uncommon whereas hyperirritability, sluggishness and progressive weakness are the usual signs of infection. The fact that suckling mice should be less responsive than adult mice to the toxic effect has a counterpart with bacterial exotoxins, since 3-day-old mice have been reported to be less responsive than adult mice to the toxin of *Clostridium botulinum*[162].

Although evidence of an incomplete cycle of multiplication was not found with NDV in early studies[56], more recent work by Granoff[59], Prince and Ginsberg[136] and Adams and Prince[3] has shown that non-infectious forms of NDV do occur. These forms may be demonstrated in Ehrlich ascites tumor cells inoculated with NDV by both electron microscopy and the fluorescent antibody technique[3,136]. This viral antigen is reported to be non-infectious, non-hemagglutinating and non-complement fixing in the presence of homologous antiserum[136]. The hyperplasia and subsequent lysis of Ehrlich ascites cells by NDV is believed to be due to the toxic effect of NDV. It is true that propagation of the virus has been reported after prolonged serial passage in ascites cell cultures, first *in vitro* and later *in vivo*[50],

although it seems unlikely that growth could have been a factor in the experiments reported above.

Ogasawara and Nakayama[119] have shown recently that non-infectious NDV can be obtained by treatment of the virus *in vitro* with sodium ethylmercurithiosalicylate and that such virus caused consolidation of the lungs and death of mice after intranasal instillation. By the use of tetraethylammonium bromide or chlorpromazine it is possible to suppress the lesion-producing factor without affecting the infectious property of NDV. These experiments indicate that the lesion-producing factor (toxin) can be separated from the infectious property and recall the results found with xerosin and influenza virus[65]. NDV has the same effect as influenza virus on the cornea of the rabbit; that is, a progressive destruction of the corneal epithelium resulting in corneal opacity[173]. It has been pointed out[173], however, that although there is no evidence of viral multiplication either by infectivity or by hemagglutination tests, one cannot rule out the development of an antigen similar to that reported by Prince and Ginsberg[136].

In recent studies with NDV and its effect on the cornea of the rabbit, Oh and Evans[122] have reported on some interesting aspects of the reaction. It was found that within one hour of the inoculation of large amounts of NDV there was some loss of endothelial cell boundaries and the formation of isolated areas of radially arranged endothelial cells, both of which increased in numbers with time. The radially arranged endothelial cells were designated endothelial rosettes by Oh and Evans[122]. Very few rosettes were formed by the inoculation of normal allantoic fluid but they were formed after the injection of compound 48/80 (a formaldehyde polymer of *p*-methoxyphenethylmethylamine), a liberator of endogenous histamine. Suppression of the lesions caused by 48/80 was most effective with the use of both an antihistamine, pryilamine methane, and an antiserotonin agent, d-lysergic acid diethylamide (LSD). The same combination was then tried and found effective in reducing the numbers of endothelial rosettes produced by NDV. The agents did not affect either the infectivity of NDV or the capacity of host cells to be infected by the virus. Oh and Evans[122] point out the available evidence does not yet allow for a complete explanation of the reactions which occur after the inoculation of NDV. It may be that the lesions are caused by the release of some substance as a result of the inoculation of the virus rather than by the virus *per se* affecting the endothelial cells. Neither histamine nor serotonin, known to be present in the tissue fluid of inflamed organs, regularly produced lesions on exposure to corneal endothelium *in vitro*. It may be, too, that the antihistamine and antiserotonin agents suppressed the lesions through some other physiologic effect than their respective effects on histamine and serotonin[122].

Newcastle disease virus also has a pyrogenic effect with an associated lymphocytopenia almost identical with that found with influenza virus[8,9,10].

A characteristic property of Newcastle disease virus is its ability in suspension to hemolyse a wide variety of erythrocytes[95]. The hemolysin is inhibited by calcium ions[29,100] and enhanced by dialysis against various substances, particularly distilled water (isotonicity being restored with sodium chloride prior to test)[60]. The choice of diluent also influences the amount of hemolysis obtained with isotonic sodium citrate giving optimal results. Enhancement of its activity occurs also after certain physical manipulations

such as sonic vibration, freezing and thawing, and osmotic shock. These latter effects are additive to those of dialysis and diluent[60]. Hemolytic activity is dependent on pH with optimum hemolysis at pH 5.9 and on incubation temperature with the optimum at 37° C. although some hemolysis occurs at as low as 18° C.[145]. Recent studies with high energy electron radiation of NDV have indicated that the *rate* of lysis of erythrocytes depends on the number of intact hemolysin units (estimated as 15 per virus particle) whereas the ability to lyse erythrocytes, given sufficient time, depends on the presence of only one intact hemolysin unit per virus[175,176]. Intestinal hemorrhage, often fatal in mice 24 hours following inoculation of large amounts of NDV, was reported by Ogasawara and Yasué[121]. Whether this reaction is caused by the hemolysin or by the other toxic effects of the virus (if indeed they are different substances) or both, is not known. Ogasawara and Yasué[121] suggested that the hemorrhage was due to the vasodilator effect of NDV, possibly acting on nerve endings since the reaction was prevented by prior doses of chlorpromazine hydrochloride or hydrocortisone.

Newcastle disease virus produces a nontransmissable cytopathogenic effect on Earle's strain L cells[171]. The changes in the cells were seen in 12 to 36 hours depending on the concentration of virus inoculated. Although the effect could not be transferred serially there was an accumulation of non-infectious hemagglutinins[171], which parallels the effects found with influenza virus and HeLa cells[80]. If only small inocula of NDV were used, no cytopathogenic effects were seen although infectious virus was formed. After 72 hours when intracellular virus titers were at their height, the cells were resistant to the toxic effects of large doses of NDV[172]. The resistant state lasted from 5 to 7 days, even though there was a decline in both intracellular and extracellular virus titers. It was found that the resistance to challenge with fresh NDV was caused by the production of non-infectious hemagglutinins by demonstrating the same resistance to challenge after the addition of non-infectious hemagglutinins to cell cultures[172]. This system, therefore, differs from the HeLa cell-influenza virus one, since the non-infectious hemagglutinin produced by influenza did not initiate interference[129].

c. *Mumps Virus.* Since mumps virus is a member of the same group of viruses as influenza and Newcastle disease viruses, it might be expected that similar instances of toxicity would have been demonstrated. However, mumps virus does not appear to possess toxic properties comparable to either of the former[96,135]. Intraocular injection of a large quantity of mumps virus into guinea pigs has been reported to cause a toxic effect, although multiplication of virus did occur on passage of the virus from guinea pig to guinea pig by this route[20].

Mumps virus was reported by Ogasawara *et al.*[120] to cause pulmonary consolidation in young mice after intranasal instillation without any evidence of viral multiplication, either by infectivity or hemagglutination tests. The reaction was prevented by a prior subcutaneous inoculation of tetraethylammonium bromide. Since this substance is a ganglion blocking agent, the authors[120] suggest that the autonomic nervous system is involved in the pathogenesis of consolidation both with mumps virus and Newcastle disease virus[119,120].

Mumps virus does have, however, an associated hemolysin which has been rather intensively studied and was reported first by Morgan, Enders

and Wagley[113]. The erythrocytes of man, chickens, and sheep are sensitive to this lysin and the reaction is inhibited by convalescent sera of mumps patients. Until recently the hemolysin was thought to have an optimum reaction temperature of 37° C. and most work has been done at this temperature. Soule *et al.*[151] have now reported that hemolysis is increased with temperatures at least up to 41° C. Heating to 50° C. for 2 hours, however, causes at least a 20-fold reduction in titer[151]. Its optimum pH range is between pH 7.0 and pH 8.0[113], somewhat higher than that found for the hemolysin of Newcastle disease virus[145]. The hemolysin can be distinguished from the hemagglutinin by its heat inactivation temperature, by its reaction temperature and by its pH range as well as by treatment with formalin or with ultraviolet irradiation[34,35]. Calcium inhibits the action of the hemolysin but does not affect the hemagglutinin[112]. Both the hemolysin and the hemagglutinin may be eluted from erythrocytes[113].

Morgan and his associates[54,114] have pointed out various similarities between mumps virus hemolysin and lecithinase A. Both substances are inhibited by methane, calcium and magnesium ions, both have a similar optimum temperature and pH for hemolytic activity, and neither are affected by potassium cyanide nor by sodium fluoride. Their main difference is in heat stability since lecithinase A is relatively heat stable[54]. Lecithin reduces the activity of the mumps hemolysin when added prior to, but not after, combination of the hemolysin with the red blood cell[114]. More recent studies have been done using chromatographic analysis[111]. It was felt that, since there are large amounts of lipoprotein and significant amounts of lecithin in the cell wall of chicken erythrocytes, the mechanism of action might be the splitting of a fatty acid from the lecithin present to produce the hemolytic substance lysolecithin. The methods used have been such that lysolecithin and other phosphatides would be detected. No lysolecithin was found, however, following lysis of chicken erythrocytes by mumps virus. The chromatographic analyses of the reaction mixtures have shown rather an alteration in a component with chromatographic properties similar to sphingomyelin which is present in normal chicken erythrocytes. Further proof that a lecithinase is not responsible for the hemolysis was pointed out by Soule *et al.*[151] who recalled the work of Turner[161] showing that sheep cells, which are hemolysed by mumps virus[113], do not contain lecithin. Concurrent microscopic studies have shown that the hemolysin causes a decrease in the thickness, rigidity, and strength of the cell wall but that hemoglobin is released without visible rupture of the cell wall. Thus it has been concluded that there is an alteration in some component essential for the structural integrity of the erythrocyte and it is of interest in this respect, as Moberly *et al.*[111] point out, that sphingomyelin is regarded as a structural lipid.

In later studies Soule *et al.*[151] suggest that the primary mechanism of hemolysis caused by mumps virus has not yet been found. While hemolysis of chicken erythrocytes results in the release of sphingomyelin from the lipoprotein of the stroma and subsequent destruction of 65% of the sphingomyelin, no such reaction occurs with human erythrocytes which are also hemolysed by mumps virus hemolysin.

Mumps virus, in high concentration, has been found to have a cytolytic effect on certain cell cultures (HeLa, monkey kidney epithelial and normal human epithelial) but not on all such

cultures since, for example, L cells are not affected[41,79,144]. The cytolytic property or cytolysin[144] appears to be closely related to the hemolysin. Titers of both are usually similar and both are more resistant to ultraviolet irradiation than is the infectious property. The cytolysin is not serially transferable. Russell and Morgan[144] have shown recently that although mumps virus is adsorbed to L cells it does not elute from them and they suggest that, as with the hemolysin, the action of the cytolysin is associated with the process of elution. The cytolytic effects on susceptible cells are first seen within 3 hours after addition of the virus and are quite pronounced in 6 hours. The changes consist of a loss of granularity in the cytoplasm, an increase in size of the nuclei, and a loss of cell membranes[144].

d. *Vaccinia and Variola Viruses.* There have been reports in the recent literature indicative of toxic phenomena associated with vaccinia virus. Scherer[146] demonstrated that suspensions of this virus are capable of causing agglutination of mouse fibroblasts (Earle's L strain) *in vitro* in 24 to 48 hours with no evidence of proliferation of the virus. This would appear to be a toxic manifestation and unrelated to hemagglutination. For example, fetal mouse lung cells also agglutinate in the presence of the virus and it has been shown that this phenomenon is independent of soluble hemagglutinin both by centrifugation methods and by its relative thermolability[25]. Centrifugation separates the virus from the soluble hemagglutinin while heating at 56° C. for 45 minutes destroys both infectivity and the toxic factor, leaving the hemagglutinin intact. The toxic factor is sedimented with the virus and is not inactivated by trypsin[25]. With the fetal mouse lung cells and high doses of vaccinia virus, cytopathogenic changes are seen in one hour with maximum damage occurring in 12 to 14 hours and with little if any evidence of virus growth. The possibility of limited growth may be eliminated by the use of sodium azide and sodium fluoride which inhibit vaccinia virus reproduction[25]. These substances do not affect, however, the toxic cytopathogenic effect of the virus. The toxic effect is more resistant to ultraviolet irradiation than infectivity[25], as is also the case with influenza virus[85]. Mayyasi[108] and Brown[26] and their colleagues have developed a method for the titration of vaccinia virus and its antibody based on the agglutination of L cells by vaccinia virus.

Cassel[31] has reported that vaccinia virus grown in Ehrlich ascites tumor *in vivo* underwent changes after six or more serial passages which resulted in a derived virus showing toxicity for normal mice when injected intravenously, an increased ability to destroy tumor cells, a shortened survival time of virus inoculated tumor bearing mice and, finally, a loss of capacity to produce hemagglutinin. The latter property has been explained, since Cassel and Fater[32] have demonstrated the presence of a vaccinia virus hemagglutination inhibitor in ascitic fluids. The increased ability to destroy tumor cells might, of course, be an increased pathogenicity for the tumor cells, as can occur with passage and need not represent a toxic effect. The toxicity of this tumor-derived virus for normal mice, however, is of interest. There seems no doubt that the toxicity *per se* is a property of the virus, since it has been proven that neither ascitic fluid nor mechanically disrupted tumor cells exert any effect in normal mice[31]. Further passage of the derived virus in embryonated eggs reduces its toxicity and it has been suggested that it

might be that the total virus per unit volume present in ascites fluid is higher than in fluids obtained from eggs[31].

The toxicity of vaccinia virus has been studied also in cell cultures of the human amion[17]. With human amion cells the inoculation of fully active vaccinia virus resulted in cytopathic changes consisting of rounding of the cells with degenerative changes in both the cytoplasm and nucleus. In 24 hours the cells were virtually all rounded. If, however, the virus was inactivated by ultraviolet light so that the virus was no longer infectious, an entirely different process ensued. The cells, within 12 to 24 hours, lost their polygonal shape and started to lengthen, stretch and curve in the same direction producing vortex-like formations. The cytoplasm only was involved. Later the cell sheet structure disappeared and there remained a net-like pattern of cells connected by extremely thin processes. The effect from inactive virus was ascribed to toxin because it was not infectious, a high concentration of virus was needed and the changes could not be serially transferred[17].

Although intravenous inoculation of vaccinia virus has no lymphocytopenic effect[42], there is evidence that the virus does exert a toxic effect on mouse and human leukocytes. Nishmi and Bernkopf[118] have described vacuolization, granulation, and clumping of the cells leading eventually to complete destruction. No multiplication of the virus occurs in such leukocyte suspensions and in fact the amount of infectious virus steadily decreases. The differentiation of the toxic effect from infectivity was made on the basis of their relative stabilities to heat and ultraviolet irradiation. Treatment with ultraviolet light for one minute resulted in a decline in the infectivity titer from $10^{7.5}$ to $10^{5.0}$ $TCID_{50}$ per ml., while the decrease in toxicity was only tenfold[118].

Although variola virus has not been studied so extensively as vaccinia virus, Hahon[69] reported on the growth of variola virus in various tissue culture systems. It was found that variola virus, in high concentration only, agglutinated L cells. The reaction occurred within 24 hours, before measurable viral propagation. With smaller inocula, variola virus grew in L cells. A detailed study of the probable toxic reaction in L cells was not reported.

e. *Equine Encephalomyelitis Viruses.* Evans and Bolin[43] reported on the corneal reaction of the viruses of Eastern and Western equine encephalomyelitis following intraocular inoculation into rabbits. Both viruses cause corneal opacity similar to that found after intraocular injection of either influenza virus or Newcastle disease virus[173]. Very large amounts of virus in terms of mouse lethal doses are required to bring about the reaction[43]. With EEE virus, for example, a 10% suspension of mouse brain, fatal for mice at a 10^{-7} dilution, must be used undiluted to elicit the toxic reaction. Although moderate growth of virus occurred in a few instances it was not felt to be a significant factor in the reaction[43]. Animals inoculated intravenously with high concentrations of WEE virus exhibit toxic symptoms[46]. The toxin causes a febrile reaction and leukopenia after intravenous inoculation in rabbits, and kills mice in 2 days after intravenous injection. Gross necrotic lesions are found in the liver in the absence of viral multiplication. The toxin is destroyed by heating at 56° C. for 30 minutes, a treatment which also markedly reduces the infectivity titer (10,000-fold). The toxin is neutralized by specific immune serum[46].

f. *Poliomyelitis Virus.* Ackermann and his associates[1,2,130] have presented evi-

dence that certain strains of poliomyelitis virus produce a toxin which can be demonstrated in cultures of HeLa cells. It has been observed that although the addition of fluorophenylalanine to the HeLa cell culture prevents viral multiplication it does not affect the cytopathogenic changes[2]. Some slight morphological changes brought about by the flurophenylalanine may be noted in control HeLa cell cultures but these are such that they may be readily distinguished from those caused by the virus. Later work has shown that there are two periods in HeLa cell cultures infected with poliomyelitis virus during which cellular damage occurs[1,130]. The first period occurs during the first 2 hours after addition of the virus; a plateau ensues until the second period between the sixth and twelfth hours which is again followed by a plateau. The second period begins at approximately the time that poliomyelitis virus is known to be released from the cells; intracellular poliomyelitis virus has been shown by Maassab *et al.*[101] to be demonstrable first between the third and fourth hours after infection, with release of extracellular virus beginning between the sixth and seventh hours. It is assumed that the first period of cellular damage is a toxic reaction *per se*. The toxic effect of poliomyelitis virus is neutralized by specific antiserum and the virus can be selectively neutralized, leaving its toxic property intact, with antiserum prepared against a different strain of the same type of poliomyelitis virus. The toxin is quite labile and after storage for 2 to 3 weeks at 4° C. it is almost inactive whereas the infectious property, measured by plaque forming ability, remains intact[1].

g. *Adenovirus.* Several workers[24,45,92,131,133,142] have demonstrated that adenoviruses produce a toxin although an earlier report by Harford *et al.*[74] had indicated that the cytopathogenic changes seen in cell cultures infected with adenoviruses might prove to be a toxic effect. Pereira and Kelly[92,131,133] in England and Ginsberg and his associates[24,45] in the United States have shown that this toxin or toxic factor causes an early cytopathogenic effect in HeLa cell cultures which is seen at 3 to 4 hours after inoculation with undiluted virus[45] or after over-night incubation with virus diluted 10^{-2} to 10^{-4} [131]. The changes are described as clumping and rounding of the cells with none of the nuclear changes usually associated with adenoviruses in cell cultures. The characteristic nuclear changes are not seen until 2 to 3 days after inoculation[24,45,131]. The toxin is inactivated by trypsin[45,131,142], but not by ribonuclease or deoxyribonuclease[45] and hence it is felt to be protein in nature[45,131,142]. The toxin is relatively heat-stable, retaining full activity after heating to 56° C. for 60 minutes. Heating to 60° C. for 15 minutes results in partial inactivation while 100° C. for 2 minutes completely inactivates it. The toxin may be separated from the virus particles by high-speed centrifugation and all the infectious activity is found in the sediment which has no toxicity. The supernatant contains the toxin in virtually full titer[45,131]. The toxin is neutralized by type-specific immune serum but not by heterologous type antiserum or normal serum[45,131]. Three antigens have been demonstrated in partially purified preparations of adenovirus which have different electrophoretic mobilities of which only one shows the early cytopathogenic or toxic effects in HeLa cell cultures[132]. Pereira and co-workers[6,97] have studied further the antigens of adenovirus by agar gel diffusion techniques[6] and chromatography on DEAE-cellulose[97]. In both cases they found one antigen,

protein in nature, responsible for the early cytopathogenic or toxic effect. Philipson[134] reported similar results using DEAE-cellulose. The antigen responsible for the early cytopathogenic effect was common to both type 2 or type 5 adenovirus, although it was more reactive with homologous antiserum.

Summary and Conclusion. The experimental work which has been summarized in this section indicates that several viruses possess a toxic factor when inoculated into a suitable test system in high concentration. Although the question of partial or incomplete cycles of viral multiplication, particularly with influenza and Newcastle disease viruses, poses a question as to whether the effect is due to this partial cycle rather than to a toxin, sufficient evidence has been presented to show that under certain conditions viral multiplication and cellular injury are not necessarily interdependent and that in many cases a toxin presents the best explanation for the observed phenomena. The toxins described are often compared with bacterial endotoxins and in a few instances with bacterial exotoxins. Their properties, however, are such that they do not fit conveniently into either group. Their close association with the viral particle, with the exception of adenovirus toxin, classifies them as endotoxins but their heat lability, toxoiding after the addition of formalin and their specificity distinguishes them from the bacterial endotoxins. The pyrogenic effects of influenza and Newcastle disease viruses after intravenous inoculation into rabbits present a picture different from the other viral toxins such that they resemble more closely bacterial endotoxins. Here again, however, their heat lability and the time interval after injection for the effect serve to distinguish them somewhat from the bacterial endotoxins.

In any event, with the evidence presently available, there is little doubt that the described events following the inoculation of certain viruses are caused by associated toxins.

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BOOK REVIEWS AND NOTICES

MEDICINE TODAY. A REPORT ON A DECADE OF PROGRESS. BY MARGUERITE CLARK. Pp. 360. New York: Funk & Wagnalls Co., 1960. Price, $4.95.

READERS of articles on medical science in the more cultured newspapers and magazines by now have become accustomed to the products of lay writers. However, many will be surprised by the extent to which such lay writing has been developed, as is well exemplified in the present volume. Even though dealing with a very active period, it presents a well-supported, reasonably complete, and accurate survey of the medicine of the past decade. From the pen of "one of the top ten (lay) science writers in America today," word is brought often in breezy reportorial style about the latest developments, many of which are unknown to the layman, and perhaps to physicians as well. Naturally, new forms of treatment are emphasized, and but few M.D.s, each struggling ever more frantically to keep up with the "literature" even of his special field, will fail to welcome what the author, a member of the "National Association of Science Writers," has to say. Always, the doctor's name and sufficient details are given to permit an easy check-up. The astonishing time spent by the news writer in preparation—on attending meetings and interviews with top authorities, on library research and on the polishing-up needed to satisfy the higher-ups ("Newsweek," in the case of this author)—guarantees an efficiency, which can easily surpass that of even the well-trained doctor, hard pushed by the demands of clinical practice.

The present volume deals with 13 topics, ranging from arteries to viruses, followed by 13 "diseases unconquered" (acne, allergies, multiple sclerosis, tooth decay, tuberculosis, etc.). Of the main topics, mental health appropriately requires 51 pages; child growth, 38; cancer, 37; arthritis and rheumatism, 37; the shortest being diabetes, 12 pages. Each topic had at least one or more items that were "news" to me. The journalist's freedom, aided by the broader attitude of our profession itself toward appearing in the lay press, permits free use, for instance, of living doctor's and patient's names, and an elastic style, that mingles medical history with the most recent advances in whatever way makes the best story. Conjectures for the future appear at times but never are they the reporter's own, being always based on the investigator's written or spoken word.

Properly used, then, this can serve not only "the intelligent reader" as an entertainer and eye opener, but also the hard pressed clinician, and the college boy contemplating a medical career. I know of no book that reaches into so many aspects of this complicated discipline. E.K.

THE ALKALOIDS CHEMISTRY AND PHYSIOLOGY, VOLUME VII. Edited by R. H. F. MANSKE. Dominion Rubber Research Laboratory, Guelph, Ontario. Pp. 559. New York and London: Academic Press, Inc., 1960. Price, $17.00.

CONTRIBUTORS to this second volume of supplement to a 5-volume series of reviews are: J. E. Saxton, indole alkaloids; V. Boekelheide, erythrina alkaloids; H. T. Openshaw, quinoline alkaloids other than those of cinchona and quinazoline alkaloids; N. J. Leonard, lupin alkaloids; O. Jeger and V. Prelog, steroid alkaloids of the holarrhena, solanum, and veratrum groups; J. Stanek, phthalideisoquinoline alkaloids; M. Kulka, bisbenzylisoquinoline alkaloids; E. S. Stern, diterpenoid alkaloids from aconitum, delphinium and garrya species; and R. H. F. Manske, ipecac, isoquinoline, and lycopodium alkaloids and minor alkaloids of unknown structure.

The emphasis, as in Volume VI, is on newer methods of synthesis, evidence for steric relationships, and conformational illustrations, to the almost complete exclusion of physiological data. The literature is exhaustively reviewed through 1957. Liberal use of tables makes information readily available, especially the multiplicity of names for the same alkaloid. This volume is a necessary and valuable reference for all those interested in alkaloid structure and biogenesis. M.T.

TABULATING EQUIPMENT AND ARMY MEDICAL STATISTICS. BY BRIG. GEN. ALBERT G. LOVE, USA (RET.), COL. EUGENE L. HAMILTON, MSC, USAR, AND IDA LEVIN HELLMAN, M.SC. Pp. 202; 36 ills. Washington: Office of the Surgeon General, Department of the Army, 1958. Price, $2.00.

THE development of medical records in the U.S. Army is traced in this monograph. The

application of records to individual patient service function and to medical statistics, as well as the new information derived from their analysis are discussed in historical perspective.

The medical statistics program of the Army has had important implications for military personnel and policies during its 70 years' development. Its considerable impact on civilian programs has been generally less appreciated.

This volume will be of especial interest to statisticians, hospital registrars and librarians, public health workers, and others concerned with the collection and application of biostatistical data. C.Z.

THE HEAD, NECK, AND TRUNK. MUSCLES AND MOTOR POINTS. By DANIEL P. QUIRING, PH.D., AND JOHN H. WARFEL, PH.D., Late Head of the Anatomy Division, Cleveland Clinic Foundation and Assistant Professor of Anatomy, The University of Buffalo, School of Medicine. 2nd ed. Pp. 124; 109 ills. Philadelphia: Lea & Febiger, 1960. Price, $3.25.

THIS book is a study aid for the medical student to be used in conjunction with Gray's and Cunningham's textbooks of anatomy. Each muscle is described by a clear diagram and concise statements that give its course, origin, insertion, function, nerve and blood supply. References to the above mentioned classics are added.

The authors' search for brevity of expression leads to statements that may baffle the beginner. For instance, the often repeated term "rotation" omits reference to the direction of rotation. Also, "compresses abdomen" is given, without qualification, as a function of the pyramidalis muscle.

In reading this beautifully executed second edition, one regrets that it was not brought closer to medical education's present day emphasis on functional concepts of anatomical structures. The addition of diagrams designed to give the student an understanding of the functions of muscles would have enhanced the value of this useful book for student and practitioner, alike. S.Z.

HORMONE. EINFUEHRUNG IN IHRE CHEMIE UND BIOLOGIE. By OLDRICH HANC, Forschungs Institut fer Pharmazie und Biochemie Prag. With a chapter on the analysis of steroid hormones in biological material by O. SIBLÍKOVÁ, Prag. Pp. 618; 9 ill. and 6 plates. Jena: Gustav Fischer Verlag, 1959. Price, DM 40.

THIS is a German translation of the second revised Czech edition. The arrangement of the material follows conventional lines. There is an introductory chapter, dealing amongst others with definition and classification of hormones, methodology of hormone research and hormones of invertebrates. This is followed by chapters on the individual hormones. In each of these chapters chemistry, biosynthesis intermediary metabolism of the hormone, preparation from natural sources, and synthesis is presented, followed by a brief discussion of the biological properties and physiologic actions. There is an additional chapter on hormones and enzyme systems, and a last sketchy chapter on hormones and malignant tumors. The book is readable. One wonders, however, whether detailed data on hormone preparation and isolation from glandular extracts really has a place in a book which, in view of its scope, has otherwise to keep to a textbook level. Both American and European literature is quoted. K.P.

RADIOGRAPHIE DU CRANE ET DE LA FACE DANS LA MALADIE OSSEUSE DE PAGET. By J. A. LIEVRE ET H. FISCHGOLD, Hopitaux de Paris. Pp. 132; 75 ills. Paris: Masson et Cie, 1959. Price, 4,000 fr.

THE subject of Paget's disease of the skull and face has been handled by the authors in a compact but detailed manner. The material for the book has been obtained from 389 cases of Paget's disease studied from 1947 to 1957. Seventy-five illustrations were selected to show the Roentgen findings (the basic densities, osteoporosis circumscripta, enlargement of the vault, vascular changes) and to demonstrate special techniques (planigraphy of the lateral skull, tangental views). The evolution of the disease process is shown by serial studies of a patient followed for 11 years.

The illustrations are excellent but are positives. H.W.

ADVANCES IN PROTEIN CHEMISTRY, VOLUME 14. Edited by C. B. ANFINSEN, JR., M. L. ANSON, KENNETH BAILEY, AND JOHN T. EDSALL. Pp. 520; illustrated. New York: Academic Press, Inc., 1959. Price, $14.00.

THIS is the latest volume in a distinguished series of reviews dealing with advances in protein chemistry. Included in the present publication are the following: Some Factors in the Interpretation of Protein Denaturation, by W. Kauzmann; The Specificity of Protein

Biosynthesis, by M. Vaughan and D. Steinberg; Zone Electrophoresis in Starch Gels and Its Application to Studies of Serum Proteins, by O. Smithies; Structural Aspects of Tobacco Mosaic Virus, by H. Fraenkel-Courat and L. K. Ramachandran; The Serum Proteins of the Fetus and Young of Some Mammals, by R. A. Keckwick; The Sulfur Chemistry of Proteins, by R. Cecil and J. R. McPhee; and Biological Properties of Poly-α-Amino Acids, by M. Sela and E. Katchalski.

In addition, there is an interesting biographical article on the late Kaj Ulrik Linderstrøm-Lang who contributed so importantly to the field of protein chemistry.

Aside from its general interest to protein and other biochemists, this book will have sections of particular value to geneticists, virologists, and hematologists. C.Z.

THE LIFESPAN OF ANIMALS. Edited by G. E. W. WOLSTENHOLME, O.B E., M.A., M.B., B.CH., AND MAEVE O'CONNOR, B.A. Pp. 324; 58 ills. Boston: Little, Brown and Co., 1959. Price, $9.50.

THE proceedings of the fifth in a series of Symposia on problems of Ageing are reproduced in this volume. Among the subjects discussed are: Actuarial aspects of human lifespans; parental age effects on man; onset of disease and the longevity of rat and man; and arteriosclerosis in birds. Other papers deal with the lifespan of horses, cattle, fish, bees and other insects.

Workers in medicine and biology who are concerned with studies of ageing phenomena as they relate to lifespan will find much of interest in these presentations. In addition, the current volume contains a useful cumulative index to all 5 publications in this series. C.Z.

INJURIES OF THE BRAIN, SPINAL CORD AND THEIR COVERINGS. Edited by SAMUEL BROCK, New York University. 4th ed. Pp. 739; 90 ills. New York: Springer Publishing Company, Inc., 1960. Price, $18.50.

THE first edition of this volume appeared in 1940. The book is divided into 25 chapters by 31 contributors, all authorities in their fields. Entirely new chapters have been added covering the treatment of post-traumatic meningitis and of the bladder and related complications in the spinal paraplegic.

The editor has omitted the chapter on legal aspects of injuries of the brain and spinal cord because that subject requires a monograph of its own.

There are new chapters on pathological considerations of brain injury, intracranial hematomas, on the neurosis following head injury and on the role of roentgenography in this field.

This new volume will be of great value to all treating injuries of the brain and spinal cord.

An effort should be made by the publishers to reduce its price because it deserves a wide circulation. M.S.

SIGNIFICANT TRENDS IN MEDICAL RESEARCH. Edited by G. E. W. WOLSTENHOLME, O.B.E., M.A., M.B., M.R.C.P., CECILIA M. O'CONNOR, B.Sc., and MAEVE O'CONNOR, B.A. Pp. 356; 41 ills. Boston: Little, Brown and Co., 1959. Price, $9.50.

THIS volume contains the transactions of a special symposium arranged to celebrate the tenth anniversary of the Ciba Foundation. The presentations considered a wide variety of topics of contemporary interest in medical research. A partial list of the contents will suffice to illustrate the fundamental nature of the discussions: Molecular structure in relation to biology and medicine (by L. Pauling); Chemical basis of viral multiplication (by G. Schramm); Population dynamics of body cells (by M. Burnet); Genetics and medicine (by J. Waldenström); Research in chronic pulmonary disease (by D. W. Richards); Malignant transformation: its mechanisms and nature (by A. Haddow); and Research in clinical nutrition (by J. F. Brock).

The various articles and pertinent discussions offer worthwhile reading not only for those devoted to research in the basic sciences but also for clinicians and clinical investigators. C.Z.

HELP-BRINGERS: VERSATILE PHYSICIANS OF NEW JERSEY. BY FRED B. ROGERS, M.D., Temple University School of Medicine, Philadelphia, Pa. Foreword by HENRY A. DAVIDSON, M.D., Editor, Journal of the Medical Society of New Jersey. Pp. 125; 12 ills. New York, Washington, Hollywood: Vantage Press, Inc., 1960. Price, $2.95.

MEDICAL New Jersey should indeed be grateful to the author for this booklet and to his father, Lawrence H. Rogers, M.D., the third physician generation in the Delaware Valley, for his example and inspiration. This anthology of 12 New Jersey physicians, presented in chronological order from 1667

to 1917, portrays men who attained eminence in varied lines: religion, elected offices and literary activities, as well as in the practice of medicine.

Dr. Rogers' versatile and energetic studies, together with his facility in expression, have livened these pages with so many interesting sidelights that even a non-Jerseyman will be rewardingly entertained. E.K.

GLUTATHIONE. *Edited* by E. M. CROOK. Pp. 116; 17 ills. New York: Cambridge University Press, 1959. Price, $4.50.

THIS volume consists of the papers presented at a Biochemical Society Symposium (No. 17) on glutathione. Considered, in turn, were the chemistry and biochemistry of glutathione; techniques for determining it in animal tissues; enzyme systems associated with the oxidation and reduction of glutathione in plant tissues; glutathione metabolism in animals; glutathione and neural tissues; glutathione and its analogs in the lens; and thiols and radiation damage.

Workers in many areas of biochemical and biological research will find these articles to contain much valuable and interesting information regarding this fundamental substance. C.Z.

AN MMPI HANDBOOK: A GUIDE TO USE IN CLINICAL PRACTICE AND RESEARCH. Edited by W. GRANT DAHLSTROM AND GEORGE SCHLAGER WELSH, University of North Carolina. Pp. 393; 57 ills. Minneapolis: University of Minnesota Press, 1960. Price, $8.75.

THE authors intend to provide "for the beginning clinician a clear and digestible indoctrination into the methods of administering, scoring and interpreting the MMPI." The chapters on administration and scoring are brief but explicit. Succinct accounts of the tests construction are offered, specifying their derivation, the criterion and control groups used. Various interpretive approaches form a major portion of the volume. Particularly fortunate for the student is the inclusion of profiles and interpretations which nicely exemplify the reasoning processes involved. The remainder of the text is an ambitious review of its application to such problems as selection, symptom formation, emotional control, planning and assessing treatment. The quantity of research covered presents more than a cursory mention of many references. Much statistical data and the 213 newer scales derived from the MMPI are included in the appendices. The authors have succeeded in their aim of producing a sound teaching aid. M.B.

ANTITHROMBOTIC THERAPY. BY PAUL W. BOYLES, M.D. Pp. 131; 24 ills. New York: Grune & Stratton, Inc., 1959. Price, $5.00.

IN this compact monograph the author has undertaken, with conspicuous success, to provide clinicians with a practical understanding of the normal mechanisms of blood coagulation and of the coagulation defects produced by antithrombotic therapy.

It may be recommended to all clinicians who wish to obtain a concise summary of the present status of these two complex areas of medicine. C.Z.

KURZER ATLAS FÜR DAS KLINISCHE LABORATORIUM. BY DR. MED. EBERHARD GOETZE. Pp. 40; illustrated, mostly in color. Jena: Gustav Fischer Verlag, 1959. Price, DM 12.75.

THIS is a small but beautifully illustrated atlas for the clinical laboratory. Photomicrographs of normal and abnormal cells of the peripheral blood and bone marrow, and of the findings in urinary sediment, sputum, and feces are shown. The reproductions are of exceptional clarity. The descriptive text is in German. C.Z.

THÉRAPEUTIQUE CHIRURGICALE DES ICTÈRES PAR RÉTENTION. BY P. GOINARD, Professor of Surgical Therapeutics of the Faculty of Medicine d'Alger and G. PÉLISSIER, Surgical Assistant of the Hospital of d'Alger. Pp. 210; 82 ills. Paris: Masson et Cie, 1959. Price, 3,200 fr.

THIS volume is divided into two sections. The first deals with the indications for and the physiologic basis of surgical therapy. The second deals with surgical technique, including anesthesia and pre- and postoperative care. In the first section there is a discussion of all the various types of obstructive phenomena, including a group of excellent cholangiograms. The operative techniques of the methods of relieving these various obstructions are adequately illustrated in the second section.

Most of the opinions expressed are generally acceptable and most of the operations illustrated are standard procedures in this country. The operations of sphincterotomy and sphincteroplasty appear to be rated more highly by the authors than by the majority

of American surgeons. Of more than passing interest also is the section illustrating the various techniques employed by the authors in attempting to drain the bile into the intestinal tract when the common duct has been completely destroyed previously.

J.J.

DISEASES OF THE SKIN. BY JAMES MARSHALL, M.D. University of Stellenbosch Medical School. Pp. 944; 496 ills. Edinburgh and London. E. & S. Livingstone, Ltd. (The Williams & Wilkins Co., Baltimore, U.S. Agents), 1960. Price, $15.00.

THIS larger than usual "small" book on Diseases of the Skin, is intended "to provide a text that undergraduate and general practitioners may use for reference and that will serve as introductory reading for postgraduate students." It is a good book for the practitioner and student but it is doubtful whether it is detailed enough for postgraduate students. Although the work comes from Africa, the author rightfully recognizes the universality of the basic aspects of diagnosis and management of skin diseases. He also gives more than usual recognition to the importance of histopathologic examination in the investigation of dermatoses. The text is well-written and a term new to the reviewer, "Lucite," is used for any disease in which actinic rays play a major part in the cause. The book covers the entire gamut of dermatology with sections on anatomy, basic changes, general principles of diagnosis and principles of treatment. Largely orthodox classification of diseases is used. The illustrations are generally good. The text is indexed and a selected bibliography is appended for further study.

H.B.

HUMAN BIOCHEMICAL GENETICS. BY H. HARRIS. Pp. 310; 69 ills. New York: Cambridge University Press, 1959. Price, $7.00.

ADVANCES in the science of human genetics are being made at an accelerated pace. This is true for all areas in the field, but especially so for the biochemical aspects. For this reason, this excellent book is particularly timely. The author has succeeded in producing a highly readable volume which deals, in turn, with Mendelian heredity in man; amino acid metabolism; variations in carbohydrate metabolism; human hemoglobins; blood-group substances; and plasma proteins. There is also a long chapter devoted to miscellaneous inherited disorders. In the final chapter, the problem of gene action is considered.

Physicians, biochemists, and geneticists who read this work will find it both fascinating and informative.

C.Z.

ELECTROCARDIOGRAPHY: PRINCIPLES AND PRACTICE. BY ERNEST BLOOMFIELD ZEISLER, M.D., Clinical Associate Professor of Medicine, Chicago Medical School. Pp. 374; illustrated. Chicago: Login Brothers, 1960.

IT is the author's contention that electrocardiography is unnecessarily complex mainly, if not solely, because of unsound concepts. His purpose, therefore, is to smash the concepts and destroy them, so that electrocardiography will be easy to learn, simple to apply and undertood by all. He insists that the Einthoven triangle theory is wrong and because it is wrong Wilson's central terminal is wrong. Accordingly, he pleads for the abandonment of vectorcardiography and of such electrocardiographic diagnoses like electric position, localization of myocardial infarction chamber hypertrophy and so forth. He says that empiricism should be the basis for the interpretation of electrocardiographic findings other than arrhythmias, the nature of which he determines by the Principle of Least Difficulty. On the basis of the latter, he concludes that reciprocal rhythm does not exist. His arguments are verbose and prolonged further by egregious citations of cases reported by others. Of those for whom the book is intended, the electrocardiographer will be annoyed and the medical student won't know what the author is fighting about.

J.Z.

YEAR BOOK OF MEDICINE, 1959-1960 SERIES. Edited by PAUL B. BEESON, M.D., CARL MUSCHENHEIM, M.D., WILLIAM B. CASTLE, M.D., TINSLEY R. HARRISON, M.D., FRANZ J. INGELFINGER, M.D. AND PHILIP K. BONDY, M.D. Pp. 733; 110 ills. Chicago: Year Book Publishers, Inc., 1959. Price, $8.00.

THE latest in this series of volumes consists of abstracts of articles which deal with the following general topics: 1) Infections; 2) The Chest; 3) The Blood and Blood-Forming Organs; 4) The Heart and Blood Vessels and the Kidney; 5) The Digestive System; and 6) Metabolism. The extracts and editorial comments are of the high quality which is expected from the well-known editors. However, a number of articles are omitted which, in the opinion of the reviewer, merited inclusion. Nevertheless, there is summarized a large body of information which will be appreciated by internists.

C.Z.

OFFICE DIAGNOSIS. By PAUL WILLIAMSON, M.D. Pp. 470; 407 ills. (approximately—excluding graphs). Philadelphia: W. B. Saunders Company, 1960. Price, $12.50.

THIS is an excellent and much needed book. It is a highly practical, readable textbook of medicine based on office experience. Most textbooks of medicine are written by those who have spent a major part of their medical careers in in-patient ward medicine. Such books slant the students' thinking away from the realities of office practice. The present text fills the need for a more realistic approach to the daily practice of medicine. Moreover the author is courageous enough to state his position on controversial matters with frankness and with the majority of his convictions this reviewer agrees.

It should be called to the attention of the author and the publisher that the word "none" is singular, and should be followed by a singular verb. The constant use of "none are" on almost every page was annoying to this purist. W.S.

HEMOPHILIA AND OTHER HEMORRHAGIC STATES. Edited by K. M. BRINKHOUS, M.D., Professor of Pathology, University of North Carolina and PIETRO DE NICOLA, M.D., Associate Professor, University of Pavia, Pavia, Italy. Pp. 266; 97 ills. Chapel Hill: University of North Carolina Press, 1959. Price, $7.50.

THIS volume consists of a series of articles on hemophilia and other hemorrhagic states. While primarily based on a Symposium held in Rome in September, 1958, it also includes additional material by investigators who were unable to attend the meeting itself. The topics considered are as follows: 1) Hemophilia; 2) Vascular Hemophilia; 3) Acquired "Hemophilioid" States; 4) Hageman Factor and Surface Reactions; 5) Factor X; and 6) Hemostasis and Hemorrhagic Crises.

Students of blood coagulation will find in this small book a worthwhile and contemporary summary of this complex field. C.Z.

DISEASES OF THE NEWBORN. By ALEXANDER J. SCHAFFER, M.D., Associate Professor of Pediatrics, Johns Hopkins Medical School. Pp. 845; 358 ills. Philadelphia & London: W. B. Saunders Co., 1960. Price, $20.00.

MANY newer medical texts represent little more than an altered approach to a subject well documented in current standard volumes. Not so this opus. This compendium of experience with neonatal disease by an outstanding authority is well organized and sufficiently detailed to merit a place on the bookshelves of physicians charged with the care of newborn infants. The material is, for the most part, organized according to systems. The incidence, pathogenesis, pathology, diagnosis and treatment of the disorders are discussed and reasonably well documented by time-honored and current references. As noted in the author's index, the presentation is primarily practical, but theoretical basic considerations are included in brief intelligent form. Recent advances in the field are surprisingly well represented and accurately described. Where there may be differences in opinion, the author often presents both sides of the argument. The book concludes with a pharmacopeia of the newborn period and an excellent reliable index. The style is direct and clear, although it often borders on the colloquial. This work is enthusiastically recommended as a comprehensive coverage of its subject well beyond that of the standard textbooks of pediatrics. A.B.

PATHOGENESIS AND TREATMENT OF OCCLUSIVE ARTERIAL DISEASE. Edited by LAWSON MCDONALD, The Institute of Cardiology, National Heart Hospital, London. Pp. 237; 108 ills., including charts and tables. Philadelphia and Montreal: J. B. Lippincott Company, 1960. Price, $5.00.

THIS is a collection of papers representing the proceedings of a Conference held in London on occlusive arterial disease emphasizing pathogenesis and treatment. The conference papers were divided into four sections: the first section was general and was related to pathogenesis and the remaining three sections were more specific and were related to cerebrovascular disease, coronary artery disease and peripheral vascular disease, respectively. At the conclusion of each section there are several pages of discussion. These are extremely worth-while papers and their collection in one volume affords a comprehensive coverage of the subject. A.P.

GENETICS AND CANCER. Edited by the STAFF OF THE UNIVERSITY OF TEXAS M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE. Pp. 459; 78 ills. Austin: University of Texas Press, 1959. Price, $8.50.

THE papers presented at the Thirteenth Annual Symposium on Fundamental Cancer

Research (1959), sponsored by The University of Texas M. D. Anderson Hospital and Tumor Institute are reproduced in this volume.

The subject matter is divided into 6 sections, namely, 1) Genetic Theory of Cancer Etiology; 2) Fundamental Aspects of Genetics in Carcinogenesis; 3) Gene Interaction in Neoplastic Growth; 4) The Bertner Foundation Lecture by R. E. Shope on "The Role of Virus and Host in Determining the Host Reaction to the Fibroma-Myxoma Virus Complex;" 5) Genetic Basis of Cell Resistance; and 6) Heredity and Human Cancer.

The relationship of the fundamental aspects of genetics in the etiology, progression, and inheritance of cancer is under study by many workers. This volume should be of interest not only to them, but also to investigators in other biological and biochemical disciplines. C.Z.

THE LIST METHOD OF PSYCHOTHERAPY. BY ELIZABETH SHER, ELEANOR MESSING, THEODORA HIRSCHHORN, ENIS POST, ANNETTE DAVIS AND ARTHUR MESSING. Pp. 258. New York: Philosophical Library, Inc., 1960. Price, $7.50.

SIX members of the schools offer their comments upon the "List Method of Psychotherapy."

The List method of psychotherapy named for its founder, Jacob S. List, is described as of psychoanalytic derivation. The basic principle of the entire method consists in substitution of a "program for a problem." The auxiliary tool of treatment is encouraging the patient to continue his education. Everyone of the List-trained therapists is his former patient. The warm, friendly attitude of the therapist and the milieu provided for the patient is important to the treatment.

It appears to this reviewer that the List method of psychotherapy is essentially educative. We can see no resemblance between this form of psychotherapy and psychoanalysis. M.B.

THE KINETICS OF CELLULAR PROLIFERATION. Edited by FREDERICK STOHLMAN, JR., M.D. Pp. 456; illustrated. New York and London: Grune & Stratton, Inc., 1959. Price, $5.75.

A Conference on Fundamental Problems and Technics for the Study of the Kinetics of Cellular Proliferation was held in Salt Lake City in January, 1959, under the sponsorship of the Hematology Study Section, Division of Research Grants, National Institutes of Health. The papers which were presented, many by outstanding investigators, have been made available in this volume. The material is grouped under 6 general headings: 1) The Use of Classic Morphologic Technics; 2) DNA Synthesis Stability and Degradation; 3) Studies with DNA Labels; 4) Studies on Leukocyte and Platelet Production; 5) Kinetics on the Regulation of Red Cell Production; and 6) Mathematical Considerations.

There is an enormous wealth of information in these papers, and the value of the volume is further enhanced by the inclusion of pertinent discussions at the end of the respective sections. This volume is recommended to all hematologists, as well as many biochemists, biologists, cytologists and others concerned with fundamental aspects of cellular proliferation. C.Z.

PRAKTISCHE OPERATIONSKUNDE UND INSTRUMENTENLEHRE. BY PROF. DR. MED. HABIL. HARRO SEYFARTH, Clinical Professor and Director, Orthopedic University Hospital, Rostock, Germany, and ELLY JAEGER, Superintendent, Operating Room Department, Surgical and Orthopedic University Hospital, Jena, Germany. 2nd ed. Pp. 464; 214 ills. Jena: Gustav Fischer, 1960. Price, DM 36,70.

THE first edition of this work, published in 1957, enjoyed so much popularity that a second edition soon became necessary.

The personnel of the operating room requires special training because the operative treatment differs from the general nursing care of the surgical patient. Surgeons, nurses and orderlies form a closely knit team in the operating room which only by close collaboration can achieve the optimum results. Hence, it is important that each unit receives a thorough and detailed training pertaining to its special task. This book is written to facilitate the training and to familiarize the operating room personnel with its duties.

After elucidating the ground rules (surgical principles, asepsis, antisepsis, occupational hazards) the authors describe and depict with numerous illustrations the general supply and the instruments of an operating room department, the duties of the sterile and unsterile operating room personnel, and finally the set-ups, the handling of the various instruments, and the performance of the usual and important procedures are explained. The text is well arranged, printed on fine quality paper, and contains many excellent illustrations. This book is not only recommended for those operating room attendants who can read

German, but also for surgical residents. An English translation would be most welcome.

H.M.

METABOLIC HOMEOSTASIS. By NATHAN B. TALBOT, M.D., ROBERT H. RICHIE, M.D., JOHN D. CRAWFORD, M.D., AND EDITH S. TAGRIN. Pp. 132; illustrated. Cambridge: For the Commonwealth Fund by Harvard University Press, 1959.

THIS is an excellent syllabus on metabolic homeostasis. The material is treated in 2 major sections. The first contains discussions of the basic aspects of homeostasis for water, sodium, potassium, hydrogen ion, chloride, and phosphorus. In the second section, the clinical applications of this knowledge to infant feeding, parenteral fluid therapy, and renal disorders are considered.

Clearly and concisely written, with excellent illustrative charts, this book should be most helpful to all who are concerned with the clinical care of patients. C.Z.

MISSBILDUNGEN DES MENSCHLICHEN HERZENS. By PRIV.-DOZ. DR. HEINZ BARTHEL, Chief Surgeon, City Hospital, Osterode, Harz. Pp. 240; 215 ills., some in color. Stuttgart: Georg Thieme Verlag, 1960 (Intercontinental Medical Book Corporation, New York, U.S. Agents). Price, $44.75.

MALFORMATIONS of the heart used to be academic curiosities. With the advent of corrective surgical procedures, the practical interest has become great. A basic prerequisite in this field is an understanding not only of the established malformation, but of the responsible developmental factors in the ontogenesis of the heart. The presentation of such a highly specialized topic, despite admirable lucidity and logic, would be well understood only by experts in the field, were the information conveyed by the symbolism of words alone. This limitation is altogether circumvented by the use of many, magnificent illustrations (for which much credit goes to Miss Ingrid Schaumburg of Hamburg). Thanks to the illustrative part of this monograph, not only anatomists and pathologists but cardiologically interested clinicians (pediatricians, internists, and surgeons) will be greatly benefited. Ingenious flow experiments through tubes are presented, giving consideration to torsion and pressure in their effect on the development of septum-like protrusions. While the text, of course, requires a knowledge of German, the illustrations can be successfully studied without this knowledge. There are 652 valuable references. It is recommended that American publishers take a postgraduate course with Thieme publisher in Stuttgart.

H.R.

BIOCHEMICAL PREPARATIONS, VOLUME 7. Edited by HENRY A. LARDY. Pp. 102. New York: John Wiley & Sons, Inc., 1960. Price, $5.25.

ANOTHER group of reliable methods for the preparation of valuable compounds is presented in the latest volume of this series of publications. Among the methods described are those for the preparation of n-acetylneuraminic acid, adenosine di-and tri-phosphates containing P_{32}, cerebrosides, flavin adenine dinucleotide, 3-hydroxy-1-kynurenine, and tetrahydrofolic acid. Workers in biochemical research will find this volume as helpful as its predecessors. C.Z.

ERGEBNISSE DER WIRBELSÄULENFORSCHUNG. BAND 15, DIE WIRBELSÄULE IN FORSCHUNG UND PRAXIS. A Collection of Lectures and Contributions by Thirteen Authorities in their Field. Edited by DR. HERBERT JUNGHANNS, Professor of Orthopedics, Oldenburg, Germany. Pp. 140; over 100 ills. Stuttgart: Hippokrates-Verlag, 1960. Price, not listed.

THIS monograph written in German contains 12 chapters. The first by Professor Junghanns deals with problems in the investigation and treatment of vertebral column disease. The second chapter by Doctors Degenhardt and Gruter presents the basis of the structure of the vertebral column and the effects of anoxia and Roentgen-ray. The third chapter by Dr. Mau describes enchondrial dysostosis and kyphosis of the vertebral column. Dr. Moser contributed the fourth chapter on experimental investigation of the growth elements of the spinal column. The fifth chapter by Dr. Hirsch is concerned with the pathology and mechanics of aseptic lumbar disc disease. Chapter 6 by Dr. Hartmann discusses the structure and characteristics of the intervertebral disc. Chapter 7 by Dr. Hansen describes disc degeneration in animals and Dr. Horton in Chapter 8 presents biological and biochemical studies on the intervertebral disc in man. Chapter 9 by Dr. Gutmann is devoted to the roentgenographic proof of the structure of the vertebra and vertebral column. Chapter 10 by Dr. Erdmann discusses anomalies and problems of the assimilation pelvis. The eleventh chapter

by Dr. Jensen is on clinical studies of the physiology of the back musculature and the final chapter by Dr. Bugyi is on the function of the cervical vertebra by roentgenologic methods.

This monograph contains invaluable practical and theoretical material on the pathology and structure of the spinal column and will be most welcome to a broad segment of the medical profession. The publishers should present an English translation. M.S.

EMOTIONAL MATURITY. By LEON J. SAUL, M.D., Professor of Psychiatry and Chief of the Section of Preventive Psychiatry, University of Pennsylvania School of Medicine. 2nd ed. Pp. 393; 17 ills. Philadelphia and Montreal: J. B. Lippincott Company, 1960. Price, $6.50.

FOR Saul, neurosis results essentially from parental blunders. This results in a persistence of a dependent need for parents with inevitable frustration and hostility. This dependency and hostility interact with the superego. Treatment is based on helping the patient break out of his infantile state and progress toward maturity. The great mechanisms of adaptation is the fight-flight reaction.

Dr. Saul seems to derive his views from Alexander who also emphasized that unsolved childhood conflicts impelled the individual toward unsuitable reactions in reality and unconscious fantasies are minimized. Dr. Saul also seems influenced by Adler. This is evident in his discussion of dreams. A patient dreams of being reunited with his wife and the treatment is to allow the patient to visit with his wife. Mountain climbing or flying in dreams for Dr. Saul symbolizes ambition.

The oversimplification of human behavior, the failure to deal with any limitations of our knowledge, a convincing manner that the final word in psychiatry has been achieved, will appeal to certain readers. M.B.

ANTIBIOTICS ANNUAL, 1959-1960. Edited by HENRY WELCH, PH.D., and FELIX MARTI-IBANEZ, M.D. Pp. 1034; illustrated. New York: Antibiotica, Inc., 1960. Price, $15.00.

THIS volume contains the proceedings of the Seventh Annual Symposium on Antibiotics which was held in Washington, D.C., on November 4-6, 1959. Included are more than 150 papers which deal with almost every aspect of both old and new antibiotics.

Clinicians, microbiologists, pharmacologists, and biochemists will find much of value and interest in this group of reports. C.Z.

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